Journal of Food and Drug Analysis

Volume 13 | Issue 2 Article 15

Analyzing aristolochic acids in Chinese herbal preparations using LC/MS/MS

Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005, Pages 125-131 藥物食品分析 第十三卷 第二期

Analyzing Aristolochic Acids in Chinese Herbal Preparations Using LC/MS/MS

CHIU-YU HUANG[1], MU-CHUAN TSENG[1,2] AND JER-HUEI LIN[1]*

[1.] *Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, 161-2 Kunyang St., Nangang District, Taipei City 115, Taiwan, R.O.C.*

[2.] *Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, 91 Hsueh-Shih Rd, North District, Taichung City 404, Taiwan, R.O.C.*

(Received: July 12, 2004; Accepted: February 14, 2005)

ABSTRACT

This paper introduces a newly developed method of applying liquid chromatography- tandem mass spectrometry (LC/MS/MS) to the analysis of aristolochic acids (AAs) in Chinese herbal (medicinal) preparations. Sensitive and highly accurate readings were obtained for this study using both a photodiode array (PDA) detector and tandem mass spectrometer. The following optimized conditions for LC/MS/MS were set for this study: Separation was accomplished using a reverse phase C18 column (2.1 × 150 mm, 5 μm). The mobile phase comprised 35% acetonitrile and a 65% aqueous solution containing 0.1% formic acid and 0.1% ammonium acetate at a flow rate of 0.3 mL/min with a split ratio of 1:1 into the PDA detector and the tandem mass spectrometer. MS/MS qualitative analysis was performed at 3.0 kV capillary voltage, with a collision energy level for aristolochic acid I (AA-I) of 10 eV and for aristolochic acid II (AA-II) of 12eV. The electrospray ionization source was operated in the positive mode. AA-I $[M + NH_4]^+$ ions at *m/z* 359 and AA-II $[M + NH_4]^+$ ions at *m/z* 329 were selected as precursor ions for daughter ion scanning. The characteristic daughter ion mass spectra for AA-I and AA-II were generated and studied carefully. Quantification of results was done using the multiple reaction monitoring (MRM) method. The $[M + NH_4]^+$ and $[(M + NH_4)- NH_3-CO_2]^+$ ions of both AA-I and AA-II were selected as precursor and daughter ions for the MRM analysis. MS/MS detection limits were defined as 2.0 ng/mL of AA-I, and 2.8 ng/mL of AA-II. The linear regression correlation coefficients of the calibration cure were 0.9992 of AA-I within the range of 0.02~16.00 μg/mL and 0.9988 of AA-II within the range of 0.028~22.40 μg/mL. Relative standard deviations of 0.73% and 10.44% for AA-I, and 1.38% and 6.10% for AA-II were determined for the intraday and interday tests. Recoveries for five differing concentrations of AA-I ranged from 99.0% to 106.9% and, for five differing concentrations of AA-II, ranged from 92.0% to 104.5%. Levels of AA-I and AA-II detected in the 12 commercial Chinese medicinal preparation samples ranged from 11.1 to 3376.0 μg/g and from 5.4 to 725.4 μg/g, respectively.

Key words: liquid chromatography- tandem mass spectrometry (LC/MS/MS), aristolochic acid, piromidic acid, electrospray, multiple reaction monitoring (MRM)

INTRODUCTION

Aristolochic acids, a group of structurally analogous nitrophenanthrene derivative compounds[(1)], are found primarily in plants of the species *Aristolochia* and *Asarum*[(2)]. The group's nephrotoxic and carcinogenic properties have earned for aristolochic acids increasing international attention. For example, several cases reported in the literature discussing patients experiencing end-stage renal failure following ingestion of Chinese herbal weight loss preparations containing AAs[(3-7)] noted not only induced renal interstitial fibrosis but also the development of upper urothelial malignancies in some patients, with aristolochic acid-related DNA adducts found in tissue samples[(8)]. Some aristolochic acid-containing plants of the species *Aristolochia* (e.g., *A. fanchi*, *A. manshuriensis*, *A. debil*) and *Asarum* (e.g., *A. canadese* and *A. caudatum*)[(2)] find their way regularly into traditional Chinese medicine preparations. The similar vernacular names (in Chinese) of these plants to other, non-AA containing medicinal plants make the risk of inadvertent use in medicinal preparations significant. For example, the name "fangchi" in Chinese is commonly used to refer to plants belonging to a variety of species. One, called "kuanfangchi" in Chinese, is an aristolochic acid-containing plant of the species *A. fanchi*. It bears a close resemblance to "fenfangchi," a plant of the species *Stephania tetratra* that does not contain AAs. To minimize the risk posed by improper use of AA-containing plants in Chinese medicinal preparations and the potential for subsequent serious adverse effects on consumers, our laboratory began, at the request of the Committee on Chinese Medicine and Pharmacy (CCMP, Taiwan's oversight authority for Chinese medicine), a series of studies on analytical methods that employ TLC[(9)] and HPLC to detect the presence of AAs in 15 Chinese plant medicinal preparations. Each medicinal preparation selected for this study is governed by an officially determined and enforced formula. Recognizing the high risk that long-term use of AAs pose to health, the CCMP imposed in 2003 a comprehensive ban on the use of AA-

* Author for correspondence. Tel: +886-2-26531239; Fax: +886-2-26531244; E-mail: linjerhuei@nlfd.gov.tw

containing plants of the genus *Aristolochia* and on sale of associated herbal preparations.

Several analytical methods employing TLC[10-12], LC/UV[13-15], and LC/MS[11,12] have been published in the literature. As traditional Chinese herbal preparations typically comprise multiple components blended together according to complex formulae, any analytical method selected must both permit precise separation of components in order to remove interference from target compounds and deliver sensitive and selective detection to maximize target compound identification. A problem with the TLC method is the lack of a detection method for follow-on confirmation once suspected compounds have been identified. The LC/UV method detects only AA levels above a certain level. An extended chromatographic separation time, more sophisticated mobile phase system, or other auxiliary method such as standard addition might be introduced to solve problems caused by elevated interference levels. In this paper, we introduce our experience using a combined LC/MS/MS method. This method delivered improved analysis results by combining LC separation and two types of detecting techniques (ultraviolet spectrometry and MS/MS spectrometry).

MATERIALS AND METHODS

I. *Materials*

A mixture containing 40% of AA-I and 56% of AA-II was purchased from Sigma Co. (St. Louis, USA) (lot no. 092K1249) and used as the standard. Piromidic acid, purchased from ICN Biomedicals Inc., was used as the internal standard. HPLC grade methanol and reagent grade formic acid were purchased from Ridel-deHaën (Seelze, Germany). HPLC grade acetonitrile was purchased from Labscan Co., Ltd. (Bangkok, Thailand). Reagent grade ammonium acetate was purchased from Merck (Germany). Test samples included 12 commercially available Chinese herbal medicinal preparations obtained from retail outlets in Taiwan. Each of the samples was under suspicion of AA contamination based on preliminary TLC screening results. Five species of plants, including Radix Glycyrrizae, White Atractylodes Rhizome, Radix Hedysari, Rhizoma Zingiberis, and Fructus jujube, were randomly selected from those plant samples submitted by pharmaceutical companies as part of their product registration process. These were used for the preparation of the blank sample for use in the recovery test study.

II. *Instruments and Experiment*

(I) *LC/MS/MS condition*

Analyses were carried out using a high performance liquid chromatograph (Waters 2690 Alliance) interfaced to a quadrupole mass spectrometer (Micromass Quattro Ultima) through a PEEK loop via a photodiode array detector (Waters 996) with the split ratio adjusted to 1:1. Chromatographic separation was performed on a C18 column (Zorbax Extend-C18, 150 × 2.1 mm i.d., 5μm) under an isocratic elution of a mixed solvent system (pH≒ 3.62) in a composition of 35% acetonitrile and 65% aqueous solution containing 0.1% ammonium acetate and 0.1% formic acid at a flow rate of 0.3 mL/min. A full UV spectrum was scanned from 200 to 350 nm and UV spectra obtained from analyses of standard AA-I and AA-II were recorded for reference. The chromatographic resolution (R) between AA-I and AA-II was calculated by dividing the difference in retention time by half the sum of baseline peak widths.

The mass spectrometer was fitted with an electrospray (ES) ionization source, which was operated in positive mode. Nitrogen served as both the desolvation and cone gas, with flow rates adjusted to 600 L/hr and 60 L/hr, respectively. Argon gas was employed in the collision-induced dissociation process. Source and desolvation temperatures were set to 120°C and 350°C, respectively. The range for the full ESI scan was set from 100 to 800 in *m/z*, and the range for a daughter ion scan was set from 200 to 400 in *m/z*. Data was acquired through multiple cumulating analysis (MCA). Qualitative analysis was carried out in daughter ion scan mode. *m/z* 359 and *m/z* 329 ions were selected as the precursor ions for AA-I and AA-II, respectively. Parameters involved with the MS/MS analysis such as capillary voltage, cone voltage, collision energy, etc. were optimized by a tuning procedure that directly and continually injected a concentration of 5 μg/mL of the AA standard into the ion source through a syringe pump (BAS Bee^{TM}) at 10 μL/min. The optimized capillary voltage was 3.0 kV and the optimized cone voltage was 20 V. Collision energy for AA-I was 10 eV and that for AA-II was 12 eV. Quantification was performed in multiple reaction monitoring (MRM) mode. The pairs of ions selected as precursor and daughter ions were *m/z* 359 and *m/z* 298 for AA-I, *m/z* 329 and *m/z* 268 for AA-II, and *m/z* 289 and *m/z* 271 for the piromidic acid, used as the internal standard. The cone voltage and the collision energy set for piromidic acid were 40 volts and 20 eV. Dwelling time within each monitoring channel was 0.25 sec. The full mass spectrum and the daughter ion spectrum obtained from the analysis of AA standards were appended to the library for reference (see Figures 1, 2 and 3).

(II) *Preparation of standard solutions and the calibration curve*

An accurately weighed amount of 5 mg of AAs was dissolved in 70% methanol in a 100 mL volumetric flask. The solution was diluted with 70% methanol to give standard solutions for calibration curves in the range of 0.02~16.00 μg/mL of AA-I and 0.028~22.40 μg/mL of AA-II (n = 7). Piromidic acid was added as the internal standard to attain a final concentration of 0.1 μg/mL in

Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005

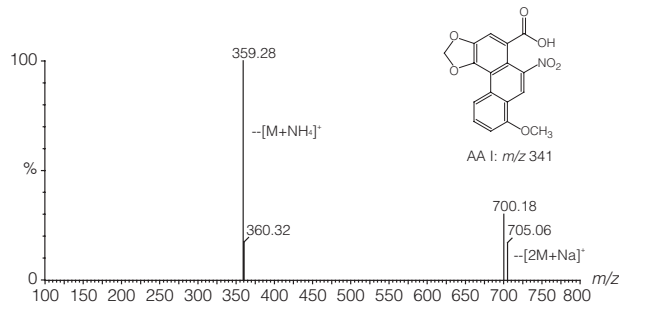

Figure 1. A full mass spectrum of AA-I from *m/z* 100 to *m/z* 800, showing considerable intensity of $[M + NH_4]^+$ ions at *m/z* 359.

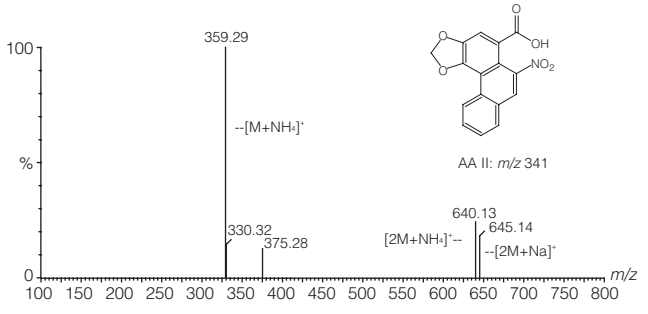

Figure 2. A full mass spectrum of AA-II from *m/z* 100 to *m/z* 800, showing considerable intensity of $[M + NH_4]^+$ ions at *m/z* 329.

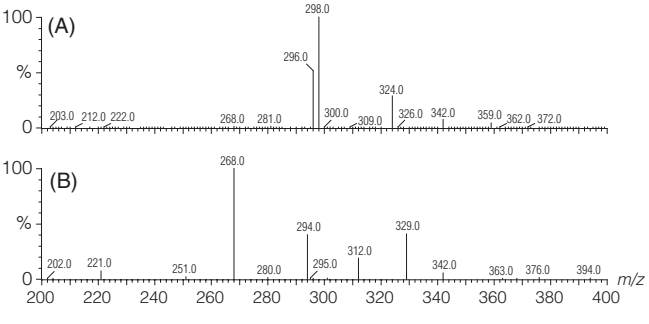

Figure 3. Daughter ion spectra of the ammonia additive precursor ions of (A) AA-I at *m/z* 359 and (B) AA-II at *m/z* 329. Distinctive fragments of de-carbon dioxide molecular ions of AA-I and AA-II were observed at *m/z* 398 and *m/z* 268, respectively.

each solution. A volume of 20 μL was injected and analyzed by LC/MS/MS with parameters described in section II. (I). Quantification was done using the MRM method and the calibration curve was obtained by plotting concentration versus peak area ratio of standard to internal standard. The linear regression equation and its correlation coefficient (*r*) were then calculated.

(III) *Sample preparation and determination*

An amount of 5 g of the samples was accurately measured and extracted with 50 mL of 70% methanol under sonication for 30 min. After filtration, 70% methanol was added to the filtrate to make the solution volume exactly 50 mL. The solution was further diluted until the final concentration of AA-I and AA-II was within the detection range of the calibration curves. Piromidic acid was added to each sample as the internal standard, giving a final concentration of 0.1 μg/mL. The injection volume was 20 μL. Qualitative analysis employing LC/MS/MS was carried out following the method described in section II. (I). Both the ultraviolet absorptive spectrum and daughter ion spectrum obtained from samples were compared against AA-I and AA-II reference spectra. The matching quality of the background subtracted daughter ion spectra between samples and the standard must be more than 80%. Further quantification of the AAs in each sample was carried out by MRM analysis, as described in section II. (I), once the presence of the AAs was confirmed. The quantities of AA-I and AA-II in each sample were calculated based on their calibration curves.

(IV) *Detection limit*

A standard sample of AAs was dissolved in 70% methanol and then diluted with 70% methanol to serial concentrations of 0.05, 0.005, 0.001, 0.0005, and 0.00005 μg/mL. We then injected 20 μL of each standard solution concentration into the LC/MS/MS and conducted a MRM analysis following the method described in section II. (I). The detection limit was defined as the point at which the peak height of the signal shown in the total ion chromatogram was three times that of noise.

(V) *Intraday and interday test*

A volume of 20 μL of the standard solution containing 0.1 μg/mL internal standard was injected six times into the LC/MS/MS over a one day period and three times each day for 5 consecutive days. Following the method described in section II. (I), MRM analysis was performed to assess the repeatability of the analysis in LC/MS/MS. Standard deviation and relative standard deviation of the peak area ratio of the standard to internal standard were then calculated.

(VI) *Recovery test*

Five differing quantities of AAs were added into a blank sample to evaluate the recoverability of MRM analysis of LC/MS/MS. The blank sample was prepared with plant materials described in section I. Referencing dosage information specified in the official formula for "Fang-Jii-Hwang-Chi-Tang" published by the CCMT (see table 1), a one-day dose of each of the plant-based ingredients in the formula, with the exception of "fangchi" (Radix Stephaniae Tetrandrae), were accurately weighed and extracted using 70% MeOH under sonication. Five different amounts of AAs were then introduced into the extract to let the final concentrations in each spiked solution attain 0.2, 0.4, 0.8, 4, and 8 μg/mL of AA-I; 0.28, 0.56, 1.12, 5.6, and 11.2 μg/mL of AA-II; and 0.1 μg/mL of piromidic acid as the internal standard. Following the MRM method described in section II. (I), analyses of AA-I,

Table 1. Official formula for "Fang-Jii-Hwang-Chi-Tang" (published by CCMT)

Ingredient	Processed Radix Glycyrrizae	White Atractylodes Rhizome	Radix Hedysari	Rhizoma Zingiberis	Fructus jujube	Radix Stephaniae Tetrandrae
One-day dose (g)	3.0	4.5	6.0	4.0	1.0	6.0

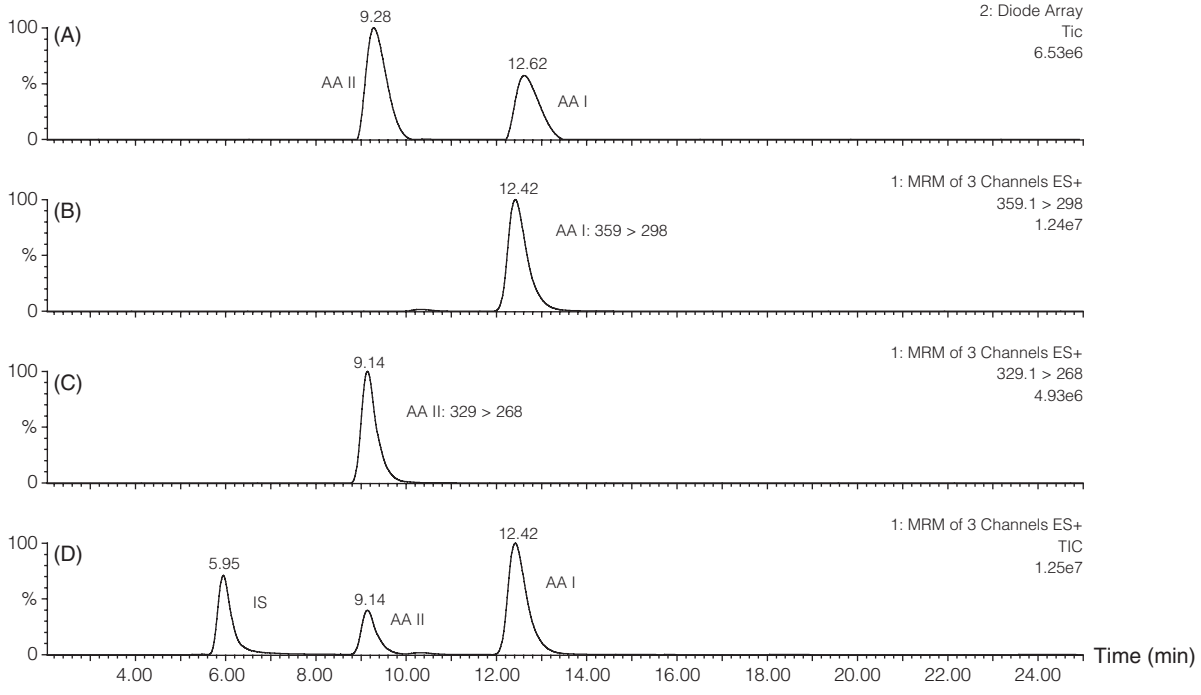

Figure 4. Chromatograms of AAs in the standard solution determined by LC/MS/MS. (A) Chromatogram of AAs detected by PDA; (B) MRM chromatogram of AA-I from *m/z* 359 to *m/z* 298; (C) MRM chromatogram of AA-II from *m/z* 329 to *m/z* 268; and (D) combined MRM chromatogram of AA-I, AA-II and piromidic acid.

and of AA-II in each spiked sample were done a total of three times. Recoveries of AA-I and AA-II were separately calculated using their calibration curves.

RESULTS AND DISCUSSION

Separating AA-I and AA-II under liquid chromatography as described in the above text is a simple operation. The two components were eluted out quickly, in approximately 12.4 min (for AA-I) and 9.1 min (for AA-II). Resolution attained 2.47. Both showed good peak shapes (Figure 4). Suitable addition of formic acid into the mobile phase to adjust the pH condition to a reasonable acidic state was successful in promoting resolution as a result of sharpened peaks. Avoiding tailing and broadening by inhibiting AA-I and AA-II from dissociation was possible because both were carboxylic compounds (Figures 1 and 2)

According to a study by Simmonds[16], the ideal ionization condition for AAs is atmospheric pressure chemical ionization (APCI) in the positive mode, including ammonium ions in the mobile phase. While two precursor ions, protonated ions ($[M + H]^+$) and ammoniated ions ($[M + NH_4]^+$) can be generated, a significant further enhancement of $[M + NH_4]^+$ ion currents occurs when either 0.1% ammonium acetate is added to the aqueous solvent (pH 7.4) or a constant infusion of 10% aqueous ammonia solution post-column is made, with the aid of a syringe pump.

As an alternative to the ion trap mass spectrometer utilized by Simmonds, we conducted a trial of the ESI^+ source by combining it with 0.1% ammonium acetate and a formic acid-containing mobile system and then operating it on a quadrupole mass spectrometer. A very intense peak of $[M + NH_4]^+$ AA-I ions at *m/z* 359 and AA-II ions at *m/z* 329 was observed in the full mass scan (Figures 1 and 2). This demonstrated the efficiency of the ionization and the applicability of the chosen precursor ions ($[M + NH_4]^+$) in this MS/MS analysis. Dimmers such as $[2M + NH_4]^+$ or $[2M + Na]^+$ also appeared in the full mass scan. Characteristic daughter ion spectra resulting from the collision-induced dissociation (CID) of AA-I and AA-II were observed under optimal experiment conditions. Both AA-I and AA-II exhibit unique daughter ion spectra (Figure 3), with a considerable intensity of $[(M + NH_4) - NH_3 - 44]^+$ ions, $[(M + NH_4) - NH_3 - 18]^+$ ions, and $[M + H]^+$ ions. $[(M + NH_4) - NH_3 - 44]^+$ ions, at *m/z* 298 for AA-I and *m/z* 268 for AA-II, appeared to be most prominent and demonstrated stability under repeated analysis. They could result from the loss of a carbon dioxide molecule in the structures and play an important role in the identification of

AAs. $[M + H]^+$ and $[(M + NH_4) - NH_3 - 18]^+$ ions were observed frequently in the MS/MS analysis, due to the loss, respectively, of one ammonia and one water molecule. Therefore, neither would be particularly useful in structure diagnosis. A strategy of having the most abundant target precursor ions occur and generating the most diagnostic daughter ions was applied in the optimization of the MS/MS condition.

Calibration curves, obtained through MRM analysis, of serial standard solutions whose concentrations were in the range of 0.05 to 40 μg/mL (corresponding to 0.02~16.00 μg/mL of AA-I and 0.028~22.40 μg/mL of AA-II) showed a positive linear relationship between concentrations and relative measured values. Linear regression equations and their correlation coefficients were:

$y = 0.4223x - 0.0749$ ($r = 0.9992$) for AA-I; $y = 0.0983x - 0.0311$ ($r = 0.9988$) for AA-II.

Detection limits for AA-I and AA-II were determined under an MRM analysis of AAs standards and were determined as 2.0 ng/mL for AA-I and 2.8 ng/mL for AA-II. These values demonstrate the high sensitivity of the LC/MS/MS method.

The level of precision achievable by the LC/MS/MS method was assessed by repeated injections six times in one day and three times each day over a five day period. The result (described in Table 2) suggested excellent repeatability of the intraday test, while detecting slight determined deviations in interday test. Slight variations in instrument-controlled variables such as desolvation gas flow and cone gas flow were observed after operation repetition over a period of days. This may have influenced inter-day results, as the tandem mass spectrometer provides highly sensitive readings. Even with these potentially influential factors, the accuracy and reliability of results should be acceptable.

Five different concentrations of AA-I (0.2, 0.4, 0.8, 4, 8 μg/mL) and of AA-II (0.28, 0.56, 1.12, 5.6, 11.2 μg/mL) were examined in the recovery study. The results determined for AA-I, ranging from 99.0% to 106.9%, and for AA-II, ranging from 92.0% to 104.5% (Table 3), suggest that an acceptable level of accuracy is achieved by the developed MRM quantitative method.

This study tested 12 samples of traditional medicinal preparations for AA-I and AA-II, with qualitative and quantitative test results shown in Table 4. Levels detected ranged from 11.1 to 3376.0 μg/g for AA-I and from 5.4 to 725.4 μg/g for AA-II. The LC/MS/MS method described in this study demonstrated superior sensitivity, specificity and selectivity over conventional TLC and HPLC methods. AA-I and AA-II exhibited typical UV spectra, with maximum absorption at 224, 250, 321 nm and 217, 251, 301 nm, respectively. Characteristic mass spectra showing prominent fragments at *m/z* 298 for AA-I and *m/z* 268 for AA-II (due to CO_2 loss in the AA structure) are also available for full daughter ion spectral comparison. These two types of spectra contribute to greater specificity in detecting AAs in plant (herbal) preparations. Compounds with the same mass-to-charge ratio were allowed to pass

Table 2. Relative standard deviations for intraday and interday analyses of AA-I and AA-II

Compound	Peak area ratio of std. to internal std. [Mean ± SD (RSD%)]	
	Intraday[a]	Interday[b]
AA-I	1.56 ± 0.01 (0.73)	9.82 ± 1.02 (10.44)
AA-II	0.43 ± 0.006 (1.38)	3.06 ± 0.19 (6.10)

[a]n = 6.

[b]n = 15, 3 injections daily for 5 consecutive days.

Table 3. Recoveries of AA-I and AA-II in the spiked sample

Compound	Spiked concentration (μg/mL)	Recovery (%) Mean ± SD (RSD%)[a]
AA-I	0.2	101.1 ± 4.45 (4.40)
	0.4	100.0 ± 1.77 (1.77)
	0.8	103.3 ± 2.87 (2.77)
	4.0	106.9 ± 2.66 (2.49)
	8.0	99.0 ± 3.25 (3.28)
AA-II	0.28	104.5 ± 2.33 (2.23)
	0.56	96.5 ± 4.41 (4.57)
	1.12	95.7 ± 4.28 (4.47)
	5.6	92.0 ± 1.43 (1.55)
	11.2	98.6 ± 3.93 (3.99)

[a]n = 3.

Table 4. Levels of AA-I and AA-II detected in 12 commercial Chinese herbal prescriptions by MRM of LC/MS/MS

No.	Form of dosage	Labeled plants	Detecting results		Amounts of AA-I (μg/g)	Amounts of AA-II (μg/g)
			AA-I	AA-II		
1	Traditional pill	Mutong	+	+	11.1	5.4
2	Traditional powder	Mutong	+	+	27.4	24.0
3	Traditional powder	Mutong	+	+	201.9	54.3
4	Traditional pill	Mutong	+	+	101.1	34.1
5	Concentrated granules	Fangji	+	+	2724.5	200.1
6	Traditional powder	-	+	+	66.5	34.7
7	Concentrated granules	Mutong	+	+	3376.0	725.4
8	Concentrated powder	Mutong	+	+	101.8	37.7
9	Concentrated granules	Madolin	+	+	297.9	43.5
10	Traditional pill	Mutong	+	+	79.8	31.5
11	Traditional pill	Mutong	+	+	41.0	25.8
12	Traditional pill	Madolin	+	+	26.1	8.8

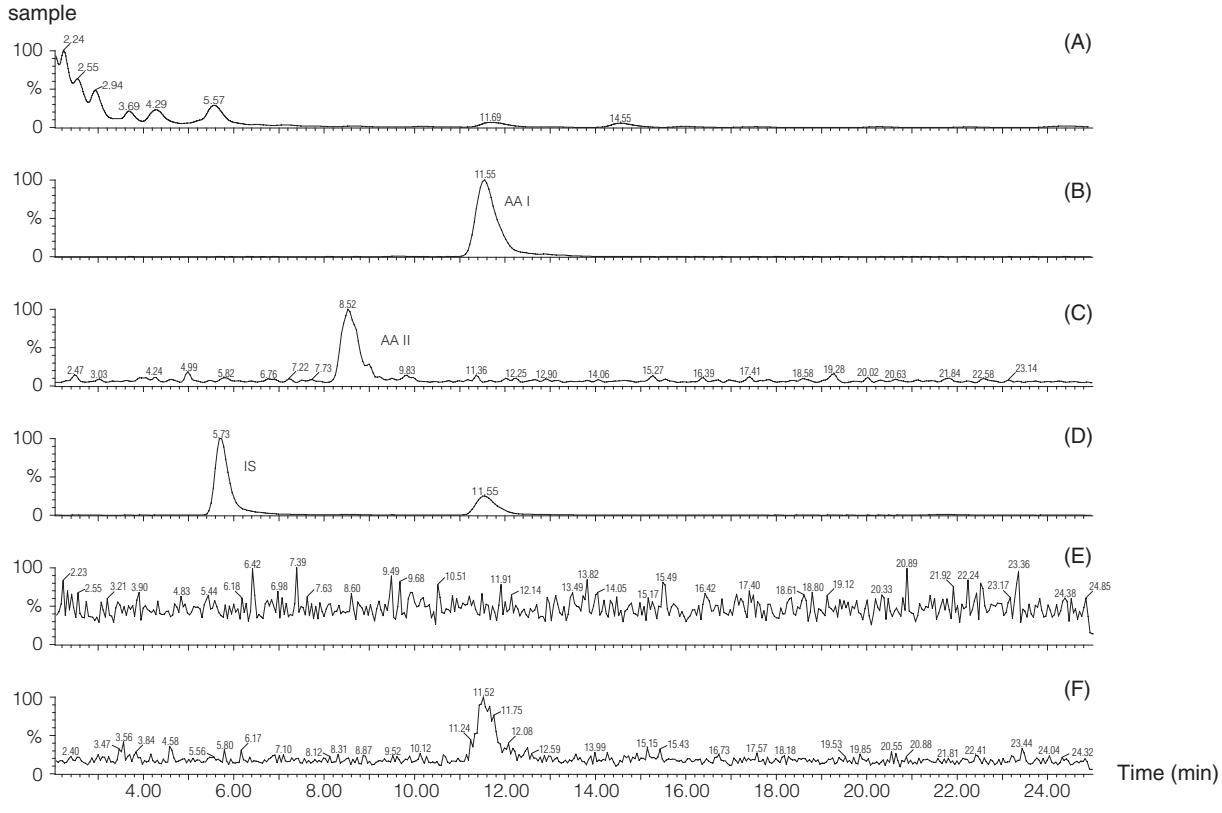

Figure 5. Chromatograms showing the analytical result of AAs in one of the 12 samples of Chinese herbal preparations by LC/MS/MS. (A) Chromatogram detected by PDA; (B) MRM chromatogram of AA-I; (C) MRM chromatogram of AA-II; (D) combined MRM chromatogram of AA-I, AA-II, and piromidic acid in the sample; (E) TIC of the daughter ion scan for the ammonia additive precursor ions of AA-II in the sample; and (F) TIC of the daughter ion scan for the ammonia additive precursor ions of AA-I in the sample.

through the first MS analyzer and undergo "collision induced dissociation" (CID), which generated characteristic daughter ions for detection by the second MS analyzer. This technique helped reduce interference from the co-eluting compounds in the sample matrix.

Figure 5 reproduces the LC/MS/MS chromatogram for sample no. 9 in Table 4. While UV signals indicating AA-I or AA-II barely register in Figure 5(A), a distinct mass message for AA-I and AA-II occurs in Figure 5(B) and (C) under MRM analysis. This shows the great difference in sensitivity between PDA detectors and tandem mass spectrometers. The enhanced sensitivity of the developed LC/MS/MS method improves AA detection capabilities significantly. Figure 5(E) and (F), respectively, display the total ion chromatogram (TIC) of the daughter ion scans for AA-I and AA-II. Although there were unclear signals of AA-II in Figure 5(E), its daughter ion mass spectrum, obtained after background subtraction, was capable of high matching quality with the AA-II reference spectrum. The developed LC/MS/MS method can also resolve problems common to TLC or LC/UV analyses related to limited LOD and interference from co-eluting compounds. This method also allows easy preparation of samples, with no clean up, purification or concentration required.

REFERENCES

1. Mix, D. B., Guinaudeau, H. and Shamma, M. 1982. The aristolochic acids and aristolactams. J. Nat. Pro. 45: 657.
2. Hashimoto, K., Higuchi, M., Makino, B., Sakakibara, I., Kubo, M., Komatsu, Y., Maruno, M. and Okada, M. 1999. Quantitative analysis of aristolochic acids, toxic compounds, contained in some medicinal plants. J. Ethnopharmacol. 64: 185-189.
3. Vanherweghem, J. L., Depierreux, M., Tielemans, C., Abramowitz, D., Dratwa, M., Jadoul, M., Richard, C., Vandervelde, D., Verbeelen, D. and Vanhaelen-Fraste, R. 1993. Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 341: 387-391.
4. Pena, J. M., Borras, M., Ramos, J. and Motoliu, J. 1996. Rapid progressive interstitial renal failure due to a chronic intake of a herb (*Aristolochia pistolochia*) infusion. Nephrol. Dial. Transplant. 11: 1359-1360.
5. Vanherweghem, J. L. 1998. Missue of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J. Altern. Complement. Med. 19: 15-20.
6. Levi, M., Guchelaar, H. J., Woerdenbag, H. J. and Zhu,

Y. P. 1998. Acute hepatitis in a patient using Chinese herbal tea- a case report. Pharm. World Sci. 20: 43-44.

7. Tanaka, A., Nishida, R., Maeda, K., Sugawara, A. and Kuwahara, T. 2000. Chinese herb nephropathy in Japan presents adult-onset Fanconi syndrome: could different components of aristolochic acids cause a different type of Chinese herb nephropathy? Clin. Nephrol. 53: 301-306.
8. Nortier, J. L., Munz Martinez, M. C., Schmeiser, H. H., Arlt, V. M., Bieler, C. A., Petein, M., Depierreux, M. F., de Pauw, L., Abramowitz, D., Vereerstraeten, P. and Vanherweghem, J. L. 2000. Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia fangchi*). N. Engl. J. Med. 342: 1686-1692.
9. CCMP, Department of Health, Executive Yuan. 2001. The identification of the crude drugs that contain aristolochic acids (Aristolochiae fangchi Radix, Aristolochiae Radix, Aristolochiae manshuriensis Caulis and Aristolochiae Fructus, ect.). Announcement No. 0900018068.
10. People's Medical Publishing House. 1988. Pharmacopoeia of the People's Republic of China (English version). p. 107. Beijing, China.
11. Loset, J. R., Raoelison, G. E. and Hostettmann, K. 2002. An LC/DAD-UV/MS method for the rapid detection of aristolochic acid in plant preparations. Planta Med. Sep. 68: 856-858.
12. Loset, J. R., Raoelison, G. E. and Hostettmann, K. 2003. Detection of aristolochic acid in Chinese phytomedicines and dietary supplements used as slimming regimens. Food Chem. Toxicol. 41: 29-36.
13. Deng, J. S., Ku, Y. R. and Chang, Y. H. 2002. Determination of aristolochic acid in traditional Chinese medicinal prescriptions containing Caulis Aristolochiae Manshuriensis by HPLC. Annu. Sci. Rep. NLFD. 20: 323-336.
14. Jou, J. H., Li, C. Y., Schelonka, E. P., Lin, C. H. and Wu, T. S. 2004. Analysis of analogues of aristolochic acid and aristolactam in the plant of Aristolochia Genus by HPLC. J. Food Drug Anal. 12: 40-45.
15. Lee, T. Y., Wu, M. L., Deng, J. F. and Hwang, D. F. 2002. High-performance liquid chromatographic determination for aristolochic acid in medicinal plants and slimming products. J. Chromatogr. B 766: 169-174.
16. Kite, G. C., Yule, M. A., Leon, C. and Simmonds, M. S. J. 2002. Detecting aristolochic acids in herbal remedies by liquid chromatography/serial mass spectrometry. Rapid Commun. Mass Spectrom. 16: 585-590.
17. Jong, T. T., Lee, M. R., Hsiao, S. S., Hsai, J. L., Wu, T. S., Chiang, S. T. and Cai, S. Q. 2003. Analysis of aristolochic acid in nine sources of Xixin, a traditional Chinese medicine, by liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry. J. Pharm. Biomed. Anal. 33: 831-837.
18. Tsai, J. L. 2001. Determination of aristolochic acid, the trace toxin in Chinese herb Xixin (Asarum spices) by liquid chromatography-mass spectrometry. Master thesis. National Chung Hsing University. Taiwan, R. O. C.