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A novel pre-column fluorescent derivatization method for the sensitive determination of aristolochic acids in medicinal herbs by high-performance liquid chromatography with fluorescence detection

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ABSTRACT

Aristolochic acids (AAs) are a family of structurally related nitrophenanthrene carboxylic acids existing in *Aristolochia*, *Bragantia*, and *Asarum* species. AAs have been proven to have nephrotoxic and carcinogenic toxicity. In this study, a novel pre-column fluorescence derivatization procedure followed by high-performance liquid chromatography-fluorescence detection (HPLC-FLD) is developed for the analysis of AAs in medicinal herbs. The nitro group in the phenanthrene ring of AAs was removed by $NaBH_4$ in water–THF (2:1, v/v), resulting in the corresponding aristolic acids. The analysis of AAs in medicinal herbs was based of the sensitive fluorescence detection of aristolic acids after the chemical derivatization. Because the produced aristolic acids are highly fluorescent the limit of detection (LOD) of AAI and AAII were lowered to 0.06 and 0.04 ng/mL, respectively, which is at least an order of magnitude lower than those in the reported HPLC and LC–MS methods. Good linearity with correlation coefficients higher than 0.997 were obtained for AAI and AII in the calibration ranges of 0.2–800 ng/mL. The derivatization conditions such as reaction temperature, time and the amount of $NaBH_4$ were optimized. The developed method provided satisfactory intra-day and inter-day precisions with RSDs less than 1.4% and 3.8%, respectively. The relative analytical error was less than 7% for the analysis of AAI and AAII in spiked matrix samples.

1. Introduction

Aristolochic acids (AAs), derived from *Aristolochia*, *Bragantia*, and *Asarum* species, is a family of structurally related nitrophenanthrene carboxylic acids, mainly consisting of aristolochic acid I (AAI) and aristolochic acid II (AAII) (Fig. 1) [1–3]. AA-containing herbs were traditionally used for the treatment of snake bites and were once promised in drug discovery and development due to its anti-inflammatory properties [4–6]. The use of herbal products containing AAs, however, is now banned in the U.S., Australia, Canada and many European and many Asian countries because of the awareness of nephrotoxic and carcinogenic toxicity [7–10]. Nevertheless, many herbal products sold on the market are still often found to be adulterated with *Aristolochia* species because of the complexity of the pharmacopoeia and the similarity of their Chinese names [11]. Furthermore, the control measures in place in many countries or regions do not cover all AAs-containing herbs. Several AAs-containing herbs (e.g., *Herba Asari* or *Xixin*) may still be sold on the market because of their "at lower dose or shorter duration" applications for anti-inflammatory and anti-virus. There is already one recent report from Taiwan about AAN after taking *Xixin* [12]. Although materials with "detectable level" of AAs may be prohibited, the detection limits of current conventional methods are too high. Prolong exposures to the AAs-containing medicinal materials, even with low contents, have induced kidney disease or cancer in Hong Kong [13].

Therefore, to minimize the risk of human exposure to aristolochic acids, development of a high sensitive and specific method for the trace analysis of AAs in herbal products is urgently required. Compared to other reported analytical methods including CE-UV [14,15], CE-ECD [16], HPLC-UV [17,18], HPLC–MS [19–21] and ELISA [22], high-performance liquid chromatography with fluorescence detection (HPLC-FLD) was shown to have better sensitivity for the determination of AAs [23,24]. Because the presence of nitro group strictly quenches the fluorescence of AAs, attempt to improve their fluorescence characteristics by pre-column derivatization procedure may play an important role in the HPLC-FLD analysis of AAs at trace levels. To the best of our knowledge, derivatization strategy was so far primarily directed at the conversion of AAs to aristolactams with a Zn/Fe-reduction reaction in acidic medium [23,24]. In this paper, a novel approach with the fluorescence derivatization of AAs is described. The non-fluorescent AAs were converted

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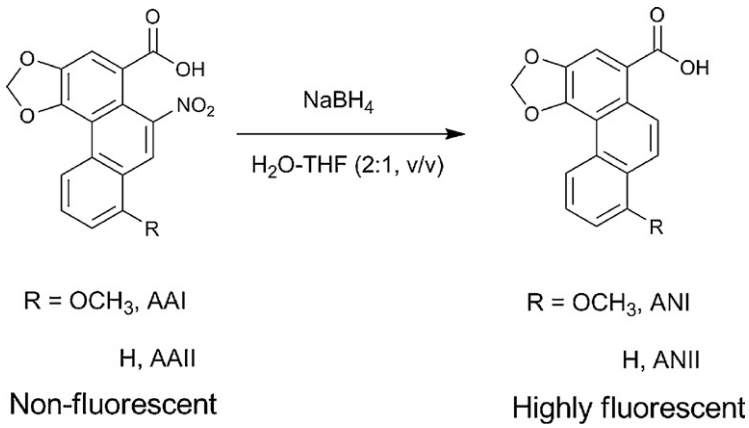

Fig. 1. Derivatization reaction of aristolochic acids (AAs) to aristolic acids with $NaBH_4$ in water–THF (2:1, v/v).

into the highly fluorescent aristolic acids, which allowed a much more sensitive determination of AAs than the existing reported methods.

2. Experimental

2.1. Chemicals and reagents

Aristolochic acid (98% purity), containing AAI 97%, was purchased from Acros (NJ, USA). Aristolochic acid II (98.75% purity) was obtained from Hong Kong Jockey Club Institute of Chinese Medicine Limited (Hong Kong SAR, China). Herbal samples *Madouling*, *Qingmuxiang*, *Tianxianteng* and *Xixin* were purchased from local Chinese medicine market with special permission. Sodium borohydride ($NaBH_4$) and anhydrous sodium sulfate (Na_2SO_4) were purchased from Acros (NJ, USA). Acetic acid and hydrochloric acid were obtained from Panreac (Barcelona, Spain).

Methanol of HPLC grade was purchased from Tedia (Fairfield, OH, USA). Tetrahydrofuran (THF) of analytical grade was obtained from Labscan (Bangkok, Thailand). Milli-Q water (18.2 MΩ) was prepared using a Milli-Q Ultrapure water purification system (Millipore, Billerica, USA).

2.2. Synthesis and characterization of aristolic acids I and II

Aristolic acids I and II were synthesized from AAI and AAII. The synthetic pathway is shown in Fig. 1. In a round-bottomed flask equipped with magnetic stirrer and condenser, a solution of AAI or AAII (20 mg) in a mixture of water–THF (3 mL, 2:1, v/v) was prepared. $NaBH_4$ (50 mg) was then added. The mixture was vigorously stirred for 1 h at 60 °C and THF was then evaporated under vacuum. The residue was acidified with hydrochloric acid to pH 4 and extracted with ethyl acetate twice. The combined ethyl acetate was washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated. The aristolic acids product was purified with recrystallization from hot ethanol. The obtained aristolic acids I and II were characterized by ^{1}H NMR and HRMS experiments. For aristolic acid I, ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.82 (1H, d, J = 9.6 Hz), 8.65 (1H, d, J = 8.8 Hz), 8.05 (1H, d, J = 9.6 Hz), 7.83 (1H, s), 7.62 (1H, t, J = 8.0 Hz), 7.24 (1H, d, J = 8.0 Hz), 6.6 5 (1H, s), 6.40 (2H, s), 4.00 (3H, s). HRMS (ESI): calcd for $C_{17}H_{12}O_5$ ([M+Na]$^+$) 319.0582, found 319.0597. For aristolic acid II, ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 13.18 (1H, s), 9.06 (1H, d, J = 9.6 Hz), 8.82 (1H, d, J = 9.6 Hz), 7.99 (1H, d, J = 9.6 Hz), 7.89 (1H, s), 7.81 (1H, d, J = 9.6 Hz), 7.70 (2H, m), 6.45 (2H, s). HRMS (ESI): calcd for $C_{16}H_{10}O_4Na$ ([M+Na]$^+$) 289.0477, found 289.0486. The purity of the synthesized aristolic acids was better than 98% from the HPLC analysis.

2.3. Preparation and derivatization of herbal samples

Individual herbal samples were powdered and sieved before use. For the analysis of individual sample, 80 mg of powder was weighed and dissolved in 2 mL 70% (v/v) methanol aqueous solution. The solution was sonicated at room temperature for 30 min and centrifuged at 13,000 rpm for 10 min. The supernatant was collected and the residue was re-extracted in the same way. The solvent extracts were combined. A portion of 1 mL of the combined extracts was transferred to an HPLC vial for the direct determination of native aristolic acids. Another portion of 2 mL of the extracts were transferred to polypropylene screw-capped glass tube for the chemical derivatization. After being concentrated to dryness under nitrogen gas stream at 40 °C, 3 mL of the mixture of water–THF (2:1, v/v) and 10 mg of sodium borohydride were added. The solution was heated at 80 °C for 15 min. After being cooled, the solution was acidified with 500 μL of 4 M hydrochloric acid and diluted to the volume of 5 mL with water–THF (2:1, v/v). The solution was centrifuged at 13,000 rpm for 5 min before the supernatant was transferred to an HPLC vial for the HPLC-FLD analysis.

For the method development and validation, combined herbs of *Tianxianteng* and *Xixin* were used as sample matrix. Similar amount of each herb powder were mixed. 80 mg of the mixed sample was extracted with 70% (v/v) methanol aqueous solution. The extract was used for the investigations of method accuracy and precision. The levels of AAs were determined before the herb matrix was used for the method validation.

2.4. Instrument and conditions

HPLC-FLD analysis was performed on a Waters Alliance 2695 HPLC system equipped with a 2475 fluorescence detector. A reverse-phase column (Agilent C18, 150 mm × 4.6 mm, 5 μm) was used with a flow rate of 1 mL/min. The mobile phase consisted of water containing 0.2% acetic acid (A) and methanol (B). The gradient elution was programmed as follows: 0–10 min, 10–50% B and 10–30 min, 50–60% B. The injection volume was 20 μL. Fluorescence detection was performed at the optimized excitation and emission wavelengths of 316 and 421 nm, respectively.

Steady-state fluorescence excitation and emission spectra were recorded on a fluorescence spectrophotometer (F-4500, Hitachi, Japan). Fluorescence quantum yield was measured using quinine sulfates as the standard (Φ_F = 0.546 in 0.5 mol L^{-1} H_2SO_4) [25]. ^{1}H NMR spectra were obtained on a NMR spectrometer (AV400, Bruker, Switzerland). High resolution mass spectrometric analysis was performed on a quadrupole time-of-flight mass spectrometer (QSTAR, Applied Biosystems, USA).

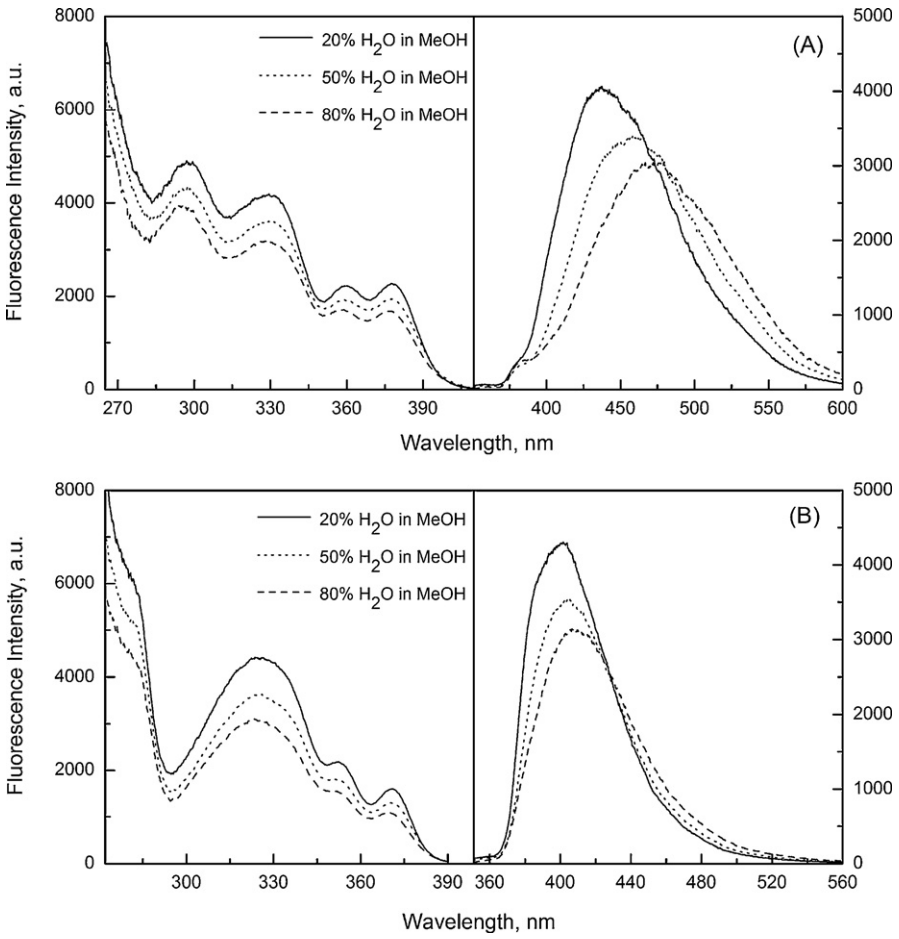

Fig. 2. Fluorescence excitation (left) and emission (right) spectra of aristolic acid I (A) and II (B) in the mixture of methanol and water containing 0.2% acetate acid. The percentage of water was increased from 20%, 50% to 80%.

2.5. Quantitative analysis of AAI and AAII

The quantitative analysis of AAI and AAII was performed after the AAs were converted to corresponding aristolic acids. The analysis with the derivatization of AAs to aristolic acids was based on the AAs calibration curves. Depending on the levels of AAs in each individual herb sample, the extract might need to be diluted and reanalyzed in order to locate the peak intensity of AAI and AAII within the dynamic calibration ranges. The calibration curve was established by using AAs standards with the same derivatization procedure as that used for the sample analysis. If native aristolic acids were detected in herbal samples, the levels of the aristolic acids converted from AAI and AAII were calculated by subtracting the background levels of aristolic acids from the total aristolic acids levels detected in derivatized samples.

For the analysis of AAs-spiked samples used in method validations, the final concentrations of AAI and AAII were calculated by subtracting the levels of AAs existing in the combined herb matrix from the total concentrations. The recovery of the entire sample preparation including the chemical derivatization was determined by the comparison with the analysis of the synthesized aristolic acids.

3. Results and discussion

3.1. Fluorescence spectra of aristolic acids I and II

The excitation and emission spectra of aristolic acids I and II in the mixture of methanol and water containing 0.2% acetate acid, in accordance with the mobile phases used in the HPLC-FLD analysis, were acquired for investigating the optimal excitation and emission wavelengths. As shown in Fig. 2, the aristolic acids exhibited a maximal fluorescence around the emission wavelength 450 and 400 nm, respectively. With increasing the content of water in methanol–water mixture from 20% to 80%, the fluorescence intensity of aristolic acids I and II was attenuated by factors of 1.35 and 1.38, respectively, accompanied by a red shift of fluorescence emission. The results suggested that higher percentage of methanol in mobile phases should be applied in order to increase the enhancement of detection sensitivity. In contrast, solvent change exerts little influence on the excitation spectra of aristolic acids I and II. Therefore, excitation wavelength at 316 nm and emission wavelength at 421 nm, instead of their maximum emission wavelength, were selected in the HPLC-FLD analysis for the simultaneous sensitive detection of the aristolic acids.

3.2. Optimization of derivatization conditions

Investigations on the derivatization conditions were performed to achieve the best yield of aristolic acids I and II. Reaction time was varied from 5, 10, 15, 30, 45, to 60 min and reaction temperature was ranged from 60, 70 to 80 °C. The results illustrated in Fig. 3 indicated that the reaction yield of aristolic acids highly depended on the reaction temperature and time. Generally, higher reaction temperature and prolonged reaction time favored the formation of aristolic acids I and II. At 80 °C, however, the yield of the aristolic acids decreased after 15 min, indicating the probable occurrence of side reaction. Therefore, the reaction conditions at 80 °C and 15 min

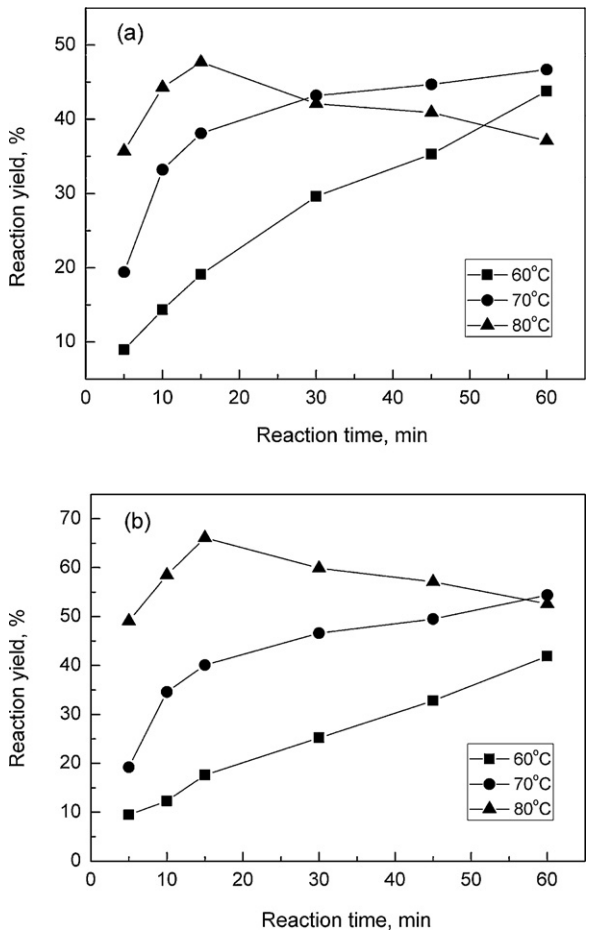

Fig. 3. Effect of reaction temperature and time on the derivatization efficiency of AAI (a) and AAII (b).

were selected. It has been reported that the herbs of *Aristolochia* species include a large number of aristolochic acid derivatives [3] that contain a nitro group in the phenanthrene ring and thus may react with $NaBH_4$. To ensure the complete derivatization of AAI and AAII in herbal samples, a 200-fold molar excess of $NaBH_4$ was used in the reaction.

3.3. Method validation

The spiked matrix samples were analyzed for evaluating the method accuracy and precision by using the developed analytical method using AAs calibration curves. AAI and AAII at different concentrations were prepared and produced aristolic acids were analyzed by using the optimized derivatization procedure and separation conditions. The peak areas of aristolic acids from the HPLC-FLD determination were plotted against the concentrations of AAI and AAII. Calibration curves with AAs concentrations from 0.2 to 800 ng/mL were separately established for AAI and AAII with correlation coefficients better than 0.997.

Limit of detection (LOD) and limit of quantification (LOQ) were obtained at levels giving signal-to-noise ratio (S/N) of 3 and 10, respectively. The LOD was 0.06 ng/mL for AAI and 0.04 ng/mL for AAII, which is an order of magnitude lower than those reported based on the reduction of aristolochic acids (AAs) to aristolactams by Zn or Fe powder in acidic medium [23,24]. The higher native fluorescence efficiency of aristolic acids than the corresponding aristolactams provided the enhanced sensitivity of HPLC-FLD analysis in the current method with the derivatization of AAs to aristolic acids. For aristolic acids I and II, Φ_F was 0.38 and 0.26. For aristolactams I and II, Φ_F was 0.17 and 0.12, respectively.

Sample preparation recovery of AAI and AAII during the sample preparation procedure including the derivatization yield of AAI and AAII to aristolic acids was evaluated. Herbal sample extracts spiked with known amounts of AAI and AAII at 0.3 ng/mL were analyzed. The produced aristolic acids or derivatization products were quantified by using the synthesized aristolic acids as the calibration standards. The levels of aristolic acids were compared to their corresponding theoretical amounts that could be calculated from the known amounts of spiked AAI and AAII for obtaining the sample preparation recovery data. The results indicated that the recovery was better than 87% for both AAI and AAII.

The method accuracy expressed by relative error was determined by adding a standard mixture of AAI and AAII to herbal sample extract at low, medium and high concentrations (Table 1). Matrix blank was analyzed prior to the experiments for method accuracy and precision. The matrix blank and matrix spiked samples were derivatized and quantified. A typical HPLC-FLD chromatogram obtained from the analysis of a derivatized matrix spiked sample with AAI at 0.29 nM and AAII at 0.32 nM is shown in Fig. 4A. Because the matrix blank contained AAI and AAII, the added levels of AAs were determined by subtracting background levels in matrix blank from the total measured concentrations. The obtained data showed that analytical error was ranged from −0.7% to +6.9% for AAI and from −3.9% to +6.3% for AAII. Intra-day precision was assessed by replicate measurements ($n = 7$) of the derivatized aristolic acids I and II within one day and inter-day precision was evaluated by analysis of the samples on seven different days. As shown in Table 1, intra-day precisions (RSD) were within 0.6–1.3% for AAI and 0.6–1.4% for AAII. Inter-day precisions (RSD) were within 2.0–3.8% for AAI and 1.4–3.5% for AAII.

The analysis of AAI and AAII in herb samples through the derivatization of AAs to aristolic acids was based on the quantitation using AAI and AAII calibration curves. Due to the available of the synthesized standards of aristolic acids or derivatization products, however, the analysis of AAI and AAII could also be performed by using the calibration curves of aristolic acids.

Table 1
Accuracy and precision for the quantitative analysis of AAI and AAII.

	Spiked level (ng/mL)	Accuracy		Precision	
		Found level [a] (ng/mL)	Rel. error (%)	Intra-day [b] (RSD, %)	Inter-day [b] (RSD, %)
AAI	0.10	0.11	+6.9	1.3	3.8
	1.5	1.49	−0.7	0.7	2.4
	15	15.6	+4.1	0.9	2.0
AAII	0.10	0.11	+6.3	1.4	3.5
	1.5	1.44	−3.9	0.8	1.4
	15	14.1	−5.8	0.6	2.3

[a] $n = 3$.
[b] $n = 7$.

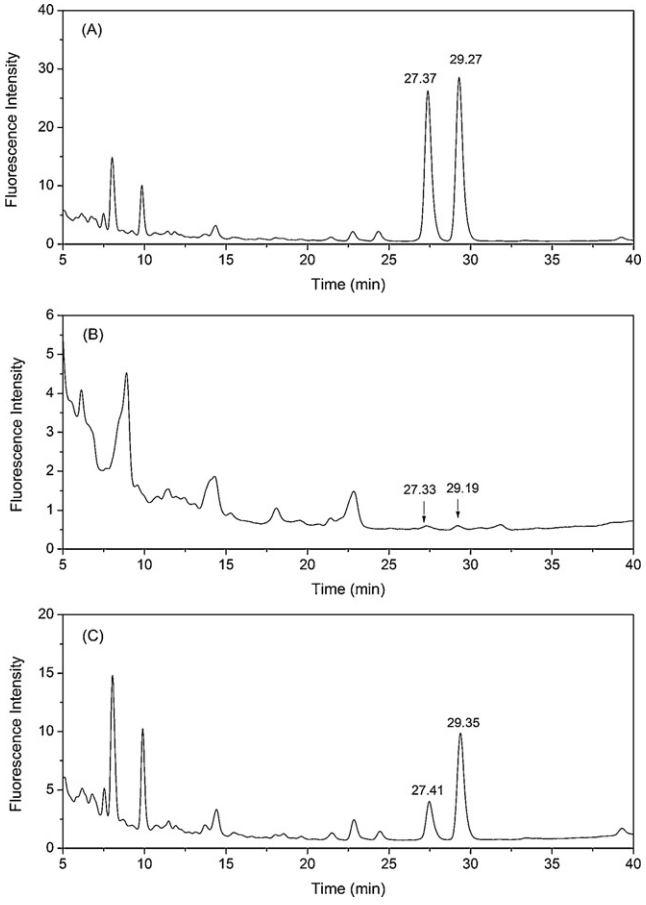

Fig. 4. Typical HPLC-FLD chromatograms of the extract of *Tianxianteng* spiked with AAI and AAII at 0.1 ng/mL (A), underivatized *Tianxianteng* extract (B) and derivatized *Tianxianteng* extract (C). The retention times of aristolic acid I and II were 29.3 and 27.4 min, respectively.

For the purpose of results comparison and further method validation, analysis of AAI and AAI were also performed by directly using the corresponding aristolic acids as the calibration standards. The synthesized aristolic acids were separated, purified and used as the calibration standards for the method validation. When the aristolic acids were used in the calibration, the obtained peak areas were plotted against the concentrations of aristolic acids. The detected levels of aristolic acids were then calculated back to those of AAI and AAII. The two analytical approaches using either AAs or aristolic acids as calibration standards gave similar results with variation of less than 12%. However, it should be pointed out that the analysis based on the calibration curves of AAI and AAII was more practical because it did not require the availability of standards of aristolic acids.

3.4. Analysis of AAI and AAII in herbal medicines

The applicability of the developed derivatization method was further investigated by analyzing real herb samples. The concentrations of AAI and AAII in four commercial herbs, namely *Madouling*, *Qingmuxiang*, *Tianxianteng* and *Xixin* were determined. Typical chromatograms obtained from the analysis of underivatized and derivatized extracts of *Tianxianteng* are illustrated in Fig. 4B and C, respectively. Compared with the retention time of aristolic acids shown in Fig. 4A, the peaks at 29.3 and 27.4 min in the herb sample analysis were attributed to aristolic acids I and II, respectively (Fig. 4B). Despite of the relatively complicated matrices, a good separation of aristolic acids I and II was achieved within 40 min on the C18 column by using methanol and water containing 0.2% acetate acid as the mobile phases. Due to the presence of the native aristolic acids in herbal samples, the concentrations of AAI and AAII were calculated by subtracting the endogenous concentrations of the aristolic acids in herbs from the total concentration of aristolic acids I and II in derivatized herbal samples. Similarly, the concentrations of AAI and AAII in other three herbs were also determined and summarized in Table 2. The successful determination of AAI and AAII in less than one gram of *Xi Xin* demonstrated the good applicability of the developed HPLC-FLD method for the trace quantification of AAI and AAII in complex herbal matrices.

Table 2
Concentration of aristolochic acids determined in the herbal samples.

	AAI, μg/g	AAII, μg/g
Madouling	289.1 ± 53.6[a]	7.9 ± 0.1
Qingmuxiang	796.2 ± 74.5	197.5 ± 14.9
Tianxianteng	8.7 ± 0.8	2.3 ± 0.2
Xixin	8.7 ± 0.1	0.58 ± 0.07

[a] $n = 3$.

4. Conclusion

A novel and simple pre-column fluorescence derivatization of non-fluorescent aristolochic acids (AAs) to highly fluorescent aristolic acids with $NaBH_4$ was developed. The analysis of AAs in medicinal herbs was based of the sensitive fluorescence detection of aristolic acids after the chemical derivatization. The high fluorescence efficiency of the produced aristolic acids allowed a more sensitive and specific analysis of AAs. The high-performance liquid chromatography-fluorescence detection method provided more than 10-fold better sensitivity that other reported HPLC and LC–MS methods [19] for the determination of AAs in medicinal herbs. The method was successfully applied for the analysis of AAs in 4 medicinal herbs including *Xixin* that contained trace levels of AAs. The quantitative results were comparable to those obtained when using the synthesized aristolic acids as calibration standards.

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References

[1] D.B. Mix, H. Guinaudeau, M. Shamma, The aristolochic acids and aristolactams, J. Nat. Prod. 45 (1982) 657–666.
[2] Y. Wang, J.X. Pan, Z.J. Jia, The advance on the research of chemical constituents and pharmacology of Aristolochia L., Nat. Prod. Res. Dev. 12 (2000) 84–94.
[3] V. Kumar, Poonam, A.K. Prasad, V.S. Parmar, Naturally occurring aristolactams, aristolochic acids and dioxoaporphines and their biological activities, Nat. Prod. Res. Dev. 20 (2003) 565–583.
[4] M.S. Kupchan, R.W. Doskotch, Tumer Inhihibitors. I. Aristolochic acid, the active principle of Aristolocic indica, J. Med. Pharm. Chem. 5 (1962) 657–659.
[5] T.S. Wu, Y.Y. Chan, Y.L. Leu, Constituents of the roots and stems of Aristolochia mollissima, J. Nat. Prod. 64 (2001) 71–74.
[6] G. Rucker, B.S. Chung, Aristolochic acids from Aristolochia manshuriensis, Planta Med. 27 (1975) 68–71.
[7] A.P. Grollman, S. Shibutani, M. Moriya, F. Miller, L. Wu, U. Moll, N. Suzuki, A. Fernandes, T. Rosenquist, Z. Medverec, K. Jakovina, B. Brdar, N. Slade, R.J. Turesky, A.K. Goodenough, R. Rieger, M. Vukeli, B. Jelakovic, Aristolochic acid and the etiology of endemic (Balkan) nephropathy, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 12129–12134.
[8] C.L. Cheng, K.J. Chen, P.H. Shih, L.Y. Lu, C.F. Hung, W.C. Lin, J.Y. Gu, Chronic renal failure rats are highly sensitive to aristolochic acids, which are nephrotoxic and carcinogenic agents, Cancer Lett. 232 (2006) 236–240.
[9] C Lebeau, V.M. Arlt, H.H. Schmeiser, A. Boom, P.J. Verroust, O. Devuyst, R. Beauwens, Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells, Kidney Int. 60 (2001) 1332–1342.

[10] U. Mengs, Arch. Acute toxicity of aristolochic acid in rodents, Toxicology 59 (1987) 328–331.
[11] C. Laing, S. Hamour, M. Sheaff, R. Miller, R. Woolfson, Chinese herbal uropathy and nephropathy, Lancet 368 (2006) 338.
[12] H.Y. Yang, J.L. Lin, K.H. Chen, C.C. Yu, P.Y. Hsu, C.L. Lin, Aristolochic acid-related nephropathy associated with the popular Chinese herb Xi Xin, J. Nephrol. 19 (2006) 111–114.
[13] S.H. Lo, K.S. Wong, V.M. Arlt, D.H. Phillips, C.K. Lai, W.T. Poon, C.K. Chan, K.L. Mo, K.W. Chan, A.Y.W. Chan, Detection of Herba Aristolochia Mollissemae in a patient with unexplained nephropathy, Am. J. Kidney Dis. 45 (2005) 407–410.
[14] S.H. Shi, W. Li, Y.P. Liao, Z. Cai, Online concentration of aristolochic acid I and II in Chinese medicine preparations by micellar electrokinetic chromatography, J. Chromatogr. A 1167 (2007) 120–124.
[15] E.S. Ong, S.O. Woo, Determination of aristolochic acids in medicinal plants (Chinese) prepared medicine using capillary zone electrophoresis, Electrophoresis 22 (2001) 2236–2241.
[16] X.G. Zhou, C.Y. Zheng, J.Y. Sun, T.Y. You, Analysis of nephroloxic and carcinogenic aristolochic acids in Aristolochia plants by capillary electrophoresis with electrochemical detection at a carbon fiber microdisk electrode, J. Chromatogr. A 1109 (2006) 152–159.
[17] J.R. Ioset, G.E. Raoelison, K. Hostettmann, Detection of aristolochic acid in Chinese phytomedicines and dietary supplements used as slimming regimens, Food Chem, Toxicol. 41 (2003) 29–36.
[18] E.S. Ong, S.O. Woo, Y.L. Yong, Pressurized liquid extraction of berberine and aristolochic acids in medicinal plants, J. Chromatogr. A 904 (2000) 57–64.
[19] J.B. Yuan, Q. Liu, G.B. Wei, F. Tang, L. Ding, S.Z. Yao, Characterization and determination of six aristolochic acids and three aristololactams in medicinal plants and their preparations by high-performance liquid chromatography-photodiode array detection/electrospray ionization mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 2332–2342.
[20] S.A. Chan, M.J. Chen, T.Y. Liu, M.R. Fuh, J.F. Deng, M.L. Wu, S.J. Hsieh, Determination of aristolochic acids in medicinal plant and herbal product by liquid chromatography–electrospray-ion trap mass spectrometry, Talanta 60 (2003) 679–685.
[21] G.C. Kite, M.A. Yule, C. Leon, M.S.J. Simmonds, Detecting aristolochic acids in herbal remedies by liquid chromatography/serial mass spectrometry, Rapid Commun. Mass Spectrom. 16 (2002) 585–590.
[22] F.Y. Yu, Y.H. Lin, C.C. Su, A sensitive enzyme-linked immunosorbent assay for detecting carcinogenic aristolochic acid in herbal remedies, J. Agric. Food. Chem. 54 (2006) 2496–2501.
[23] W. Chan, K.C. Lee, N. Liu, Z. Cai, A sensitivity enhanced high-performance liquid chromatography fluorescence method for the detection of nephrotoxic and carcinogenic aristolochic acid in herbal medicines, J. Chromatogr. A 1164 (2007) 113–119.
[24] J.B. Yuan, Q. Liu, W.F. Zhu, L. Ding, F. Tang, S.Z. Yao, Simultaneous analysis of six aristolochic acids and five aristolactams in herbal plants and their preparations by high-performance liquid chromatography-diode array detection-fluorescence detection, J. Chromatogr. A 1182 (2008) 85–92.
[25] J.N. Demas, G.A. Crosby, The measurement of photoluminescence quantum yields. Review, J. Phys. Chem. 75 (1975) 991–1024.