AUTHOR QUERY FORM

ELSEVIER	**Journal:** CHROMB **Article Number:** 17478	**Please e-mail or fax your responses and any corrections to:** **E-mail:** corrections.esnl@elsevier.thomsondigital.com **Fax:** +353 6170 9272

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
	Reference(s) given here were noted in the reference list but are missing from the text – please position each reference in the text or delete it from the list.
Q1	Tables 1 and 2 are cited in the text but not provided. Please check.
Q2	Uncited reference: This section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section.
Q3	Please provide the journal name for Ref. [40].

Thank you for your assistance.

Journal of Chromatography B, xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Analysis of urinary aristolactams by on-line solid-phase extraction coupled with liquid chromatography–tandem mass spectrometry

Su-Yin Chiang[a], Wei-Chung Shih[b], Ho-Tang Liao[b], Po-Chi Shu[a], Ming-Tsai Wey[b], Hei-Feng Huang[a], Kuen-Yuh Wu[b,*]

[a] School of Chinese Medicine, China Medical University, Taichung, Taiwan
[b] Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 13 February 2011
Accepted 29 June 2011
Available online xxx

Keywords:
Aristolochic acid
Aristolactam
Online SPE
LC/MS/MS

ABSTRACT

Aristolochic acids (AAs), nephrotoxicants and known human carcinogens, are a mixture of structurally related derivatives of nitrophenanthrene carboxylic acids with the major components being aristolochic acid I and aristolochic acid II. People may ingest small amounts of AAs from its natural presence in medicinal plants and herbs of the family *Aristolochiaceae*, including the genera *Aristolochia* and *Asarum*, which have been used worldwide in folk medicine for centuries. In order to assess AA intake, an on-line solid-phase extraction coupled with liquid chromatography–tandem mass spectrometry (on-line SPE-LC/MS/MS) method was developed to analyze their most abundant corresponding metabolites, aristolactams (ALs), in urine to serve as biomarkers. The limits of quantitation were 0.006 ng for aristolactam I (AL-I), and 0.024 ng for aristolactam II (AL-II) on column. Recovery varied from 98.0% to 99.5%, and matrix effects were within 75.3–75.4%. This method was applied to analyze ALs in the urine samples collected on days 1, 2, 4, and 7 from mice treated with 30 mg/kg or 50 mg/kg AAs. Their half lives were estimated to be 3.55 h and 4.00 for AL-I, and 4.04 and 4.83 h for AL-II, depending on AAs doses. These results demonstrated that the first simple on-line SPE-LC/MS/MS method was successfully developed to analyze urinary ALs with excellent sensitivity and specificity to serve as biomarkers to assess current AA intake from AAs-containing Chinese herbs.

1. Introduction

Aristolochic acids (AAs) are a mixture of structurally related derivatives of nitrophenanthrene carboxylic acids with the major components being aristolochic acid I (8-methoxy-6-nitro-phenanthro(3,4-d)-1,3-dioxolo-5-carboxylic acid, AA-I) and aristolochic acid II (6-nitro-phenanthro(3,4-d)-1,3-dioxolo-5-carboxylic acid, AA-II), differing from each other only by one methoxy group [1,2]. AA-I and AA-II are found in medicinal plants and herbs of the family Aristolochiaceae, including the genera *Aristolochia* and *Asarum*, which have been used worldwide as folk medicine for centuries. Pharmacological investigations have demonstrated that AA-I and AA-II are the two major active components present in plants derived from the genera *Aristolochia* [2,3]. AAs have been reported to show immunomodulatory effects in several biological systems and have been used as an immunomodulatory drug for more than 20 years in Germany [2–4]. Many formulae containing plant species of the genus *Aristolochia* are commonly used as traditional medicine to regulate menstruation, induce labor, expel parasites, relieve pain, and to treat arthritis, cancer, diarrhea, and snake-bites in East Asia, Eurasia, South America and West Africa [2–4].

AAs in medicinal plants are known to cause aristolochic acid nephropathy (AAN), a rapidly progressive interstitial nephritis that can lead to end-stage renal disease and urothelial malignancy [2,3]. AAs are suspected as one of environmental risk factors for Balkan-endemic nephropathy (BEN), a chronic renal interstitial disease characterized by a slow progression to end-stage renal disease and urothelial cancer, found endemically in several countries along the Danube river basin [2,3]. Since the outbreak of AAN in Belgium in 1993, new cases have been reported in Asian and other European countries (e.g. UK, France, Spain, and Germany) [2,3]. Carcinogenicity of AAs requires metabolic activation. Aristolactams (ALs) are the major metabolites from nitroreduction of AAs, and the cyclic aristolactam-nitrenium ions, intermediates of the reduction process, are generally considered as the ultimate carcinogens [6–10]. Therefore, ALs in urine may serve as biomarkers of AA exposure.

Although AA and AA-containing plants are classified as human carcinogens by International Agency for Research on Cancer and have been prohibited for use in the USA and the European Union

* Corresponding author. Tel.: +886 2 3366 8091; fax: +886 2 3366 8267.
E-mail address: kuenyuhwu@ntu.edu.tw (K.-Y. Wu).

1570-0232/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2011.06.045

[11–13], some herbs of genus *Asarum* are currently used in traditional medicines in Asia contain low levels of AAs. A variety of analytical methods for quantitation of AAs in herbal medicine are available, including HPLC-UV with limits of detection (LODs) ranging from sub-ppm to 10 ppb [11,14–17], HPLC-FLD with LODs at sub-ppb range [18,19], capillary electrophoresis with LODs at 10 ppb range [1,20–22], liquid chromatography coupled with mass spectrometry (LC/MS) with LODs at 10 ppb range [23–26], and ELISA method with LODs at 5 ppb range [27]. Analytical methods were also developed for the quantitation of AAs in biological samples, such as HPLC-UV with LODs of AAs at 10 ppb range in rabbit plasma [28], HPLC-FLD with LODs of AA-I at sub-ppb range in rat urine and plasma [29], CE-MS with LODs of AAs at 50 ppb range in human serum [30], and LC/MS with LODs of aristolactam I at sub-ppb range in plasma of rabbits [31]. Analysis of AA in different plants concludes that its contents could vary up to thousand-fold and AA-containing plants are not regularly consumed in many countries so that assessment of exposure to AAs is very complicated and difficult.

In order to better characterize and assess AAs exposures, analysis of urinary ALs in urine could serve as biomarkers for extremely low AA intake. But, very few analytical methods are available to analyze ALs in plasma or urine of rats [8,9,32]. To analyze the extremely low concentrations of urinary ALs due to ingestion of low amount of AAs from consuming Chinese herbs, it requires an analytical method with excellent sensitivity and specificity. In addition, on-line solid-phase extraction (SPE) can be used for sample cleanup to improve sensitivity and specificity and save labors and time in sample preparations. Therefore, the objective of this study was to develop an on-line SPE coupled with liquid chromatography–tandem mass spectrometry (on-line SPE-LC/MS/MS) to simplify sample preparation procedures and analyze urinary ALs with excellent sensitivity and specificity. This automated cleanup method was used to analyze urinary ALs to serve as the biomarkers of AAs exposures, and urinary ALs can be analyzed easily and rapidly to potentially serve as high throughput biomarkers for future epidemiology study on the potential health effects caused by the intake of AAs.

2. Materials and methods

2.1. Chemicals

Aristolochic acid sodium salts (AA-I, 65%; AA-II, 27%) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Methanol and acetonitrile were purchased from J.T. Baker (Philipsburg, NJ, USA). Zinc dust and formic acid were obtained from Riedel-de Haën (Seelze, Germany). Ammonium formate was bought from Fluka (Buchs, SG, Switzerland). Potassium phosphate was provided by Wako (Osaka, Japan).

2.2. Synthesis and purification of aristolactam I and aristolactam II

The reference standards of aristolactam I (AL-I) and aristolactam II (AL-II) were synthesized as reported [10,18]. An HPLC system (L-7000 series, Hitachi, Tokyo, Japan) equipped with a C_{18} column (250 mm × 4.6 mm, 5 μm, Supelco, Bellefonte, PA, USA) and a UV detector set at 254 nm was used to purify AL-I and AL-II. The mobile phase was consisted of 50 mM ammonium formate aqueous solution (A) and acetonitrile (B) delivered at a flow rate of 1 ml/min. The gradient started from 0% B to 55% B in 10 min and further increased to 67% B in 10 min. The eluates at the retention time of 16.7 min and 17.3 min were collected and characterized by NMR, LC/MS/MS, respectively.

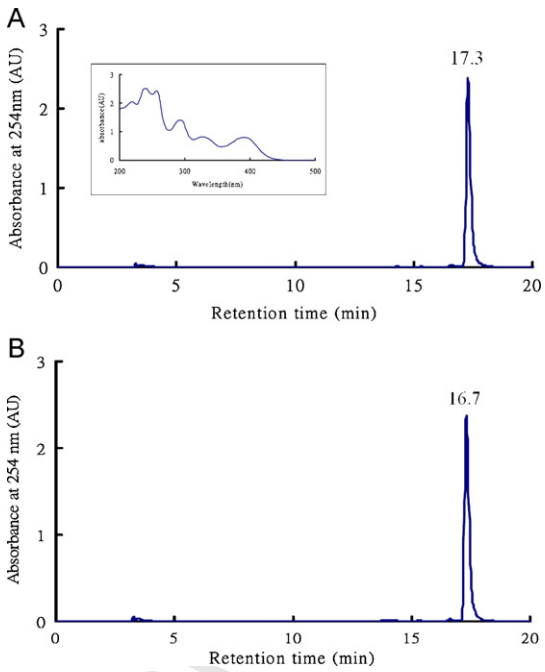

Fig. 1. Representative HPLC-UV (λ_{set} at 254 nm) chromatogram of (A) aristolactam I and (B) aristolactam II with retention time at 17.3 and 16.7 min, respectively.

2.3. On-line SPE-LC/MS/MS analysis

The on-line solid-phase extraction system consisted of an Inertsil ODS-3 cartridge (4.6 mm × 33 mm, 5 μm, GL Sciences, Tokyo, Japan), a quaternary pump, a micropump, and an autosampler (PE Series 200, Perkin Elmer, Boston, MA, USA), a two-position microelectric actuator (Valco, Houston, TX, USA) as a switching valve for process control, and an RP-18 analytical column (4.6 mm × 50 mm, 3 μm, Waters, Milford, MA, USA) for further chromatography. The scheme of the on-line SPE system was similar to that reported previously [33–36]. Sample (20 μl) was injected into the system and delivered to the extraction cartridge by the quaternary pump. Mobile phase A (10 mM ammonium formate in 10% methanol) served as a loading and washing solution at a flow rate of 1 ml/min. After 3 min, the valve was switched from loading to elution position, and the sample was eluted onto the analytical column by the micropump. The initial condition of the mobile phase was held at 25% B (0.1% formic acid in 95% methanol) for 3 min, followed by a linear gradient to 100% B in 6 min and held at 100% B for 3 min, then returned to the initial condition for 3 min. The run time cycle was 15 min for each sample. The valve was switched back to the loading position at 12 min, and the extraction cartridge was conditioned before injection of the next sample.

A triple-quadrupole tandem mass spectrometer (API 3000™, Applied Biosystems, Foster City, CA, USA) with an electrospray ionization (ESI) source was used for identification and quantitation. The multiple reaction monitoring (MRM) mode was operated to monitor the ion mass transitions for ALs. Nitrogen was used as the nebulizer gas, curtain gas, and collision-activated dissociation gas and set at 10, 12, and 12 V, respectively. The voltage of the spray needle was set at 4500 V, the turbo gas was adjusted to 8 l/min, and the temperature of the ionization source was maintained at 400 °C.

2.4. Method validation

The ALs standards were prepared by serial dilution and ranged from 1.0 to 5000 ng/ml in 50% ACN (A) and urine (B), respectively. After analysis of these standard solutions, calibration curves were

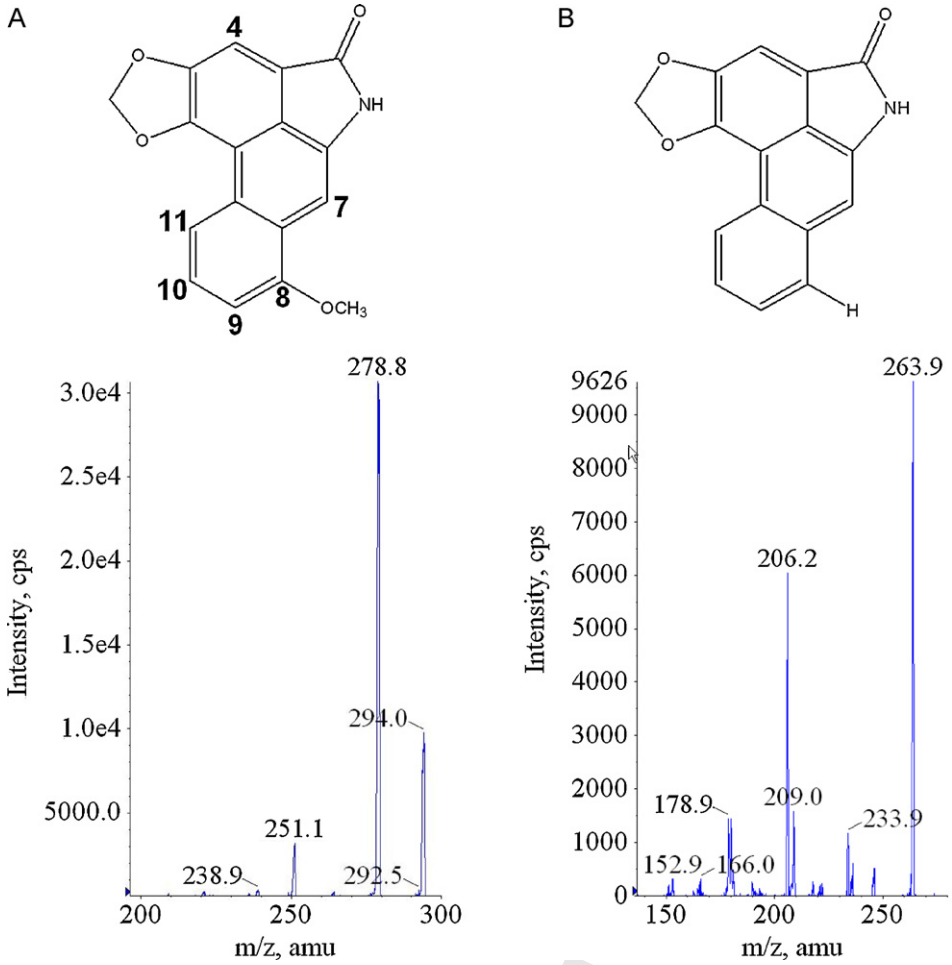

Fig. 2. Chemical structure and mass spectrum of product ion scan of (A) aristolactam I and (B) aristolactam II.

established by plotting the peak areas versus the concentrations of these standards. The recovery was determined by analysis of the A set of standard solutions with and without on-line SPE by using LC/MS/MS. To assess the influence of matrix effect, the A and B sets of standard solutions were analyzed with the on-line SPE-LC/MS/MS to separately establish calibration curves in solvent and urine matrix. The matrix effect of each analyte was determined by calculating the ratio between the slopes of the calibration curve in urine versus the slope of the calibration curve in solvent [38]. The limit of detection (LOD) and quantification (LOQ) were established at the concentrations of each analyte peak with a signal-to-noise ratio of 3 and 10, respectively.

2.5. Animal experimentation and sample pretreatment

Twenty male C3H/He mice (5–6 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The mice were bred in a well-controlled environment with periodic dark/light cycles and constant temperature (25 ± 2 °C), treated by gavage with a single dose of 30 mg/kg or 50 mg/kg of AAs, and then housed in 4 metabolic cages for each dose. There were 2, 2, 3, and 3 mice in each group. The body weights of individual mice were recorded on days 0, 3, and 6 after AA exposure. The mice were fasted during periods of urine collection, and water was given ad libitum. The urine samples were collected for 16 h on day 1, day 2, day 4, and day 7 after exposure to AAs, and animals were sacrificed for pathology examination with the order of 2, 2, 3, and 3 mice after collection of urine samples. One microliter of urine samples was aliquoted for creatinine analysis and others were stored at −20 °C until use for analysis. Urine (120 μl) was mixed with acetonitrile (120 μl) and centrifuged at 16,000 × *g* for 10 min to remove proteins. Then, samples were ready for on-line SPE-LC/MS/MS analysis. The levels of total urinary protein and creatinine were assayed on an ADVIA 1800 chemical analyzer (Siemens Healthcare Diagnostics, Madrid, Spain). The final urinary levels of AL-I and AL-II would be normalized with creatinine to adjust the potential effects of interindividual difference in water consumption.

3. Results and discussion

3.1. Characterization of ALs

Several metabolites of aristolochic acids in urine have been previously identified, including demethylated, glucuronylated and acetylated conjugations of AA-I, and ALs [8,9]. ALs are the most abundant and active metabolites of AAs [9]. Analysis of ALs may offer very valuable information to help elucidate metabolic mechanism of AAs *in vivo*. Particularly, ALs may also serve as biomarkers of AAs intake for people who may frequently consume Chinese medicines. In order to specifically analyze extremely low levels of urinary ALs to serve as biomarkers for AAs exposures, AL-I and AL-II were synthesized, purified, and characterized with HPLC-UV, LC/MS/MS, and NMR (Figs. 1–3). Fig. 1 shows that ALs peaks were collected for purification according to a representative HPLC-UV chromatograms with the retention times at 17.3 and 16.7 min for ALs-I and II (Fig. 1). The full-scan shows that the $[M+H]^+$ ions of AL-I

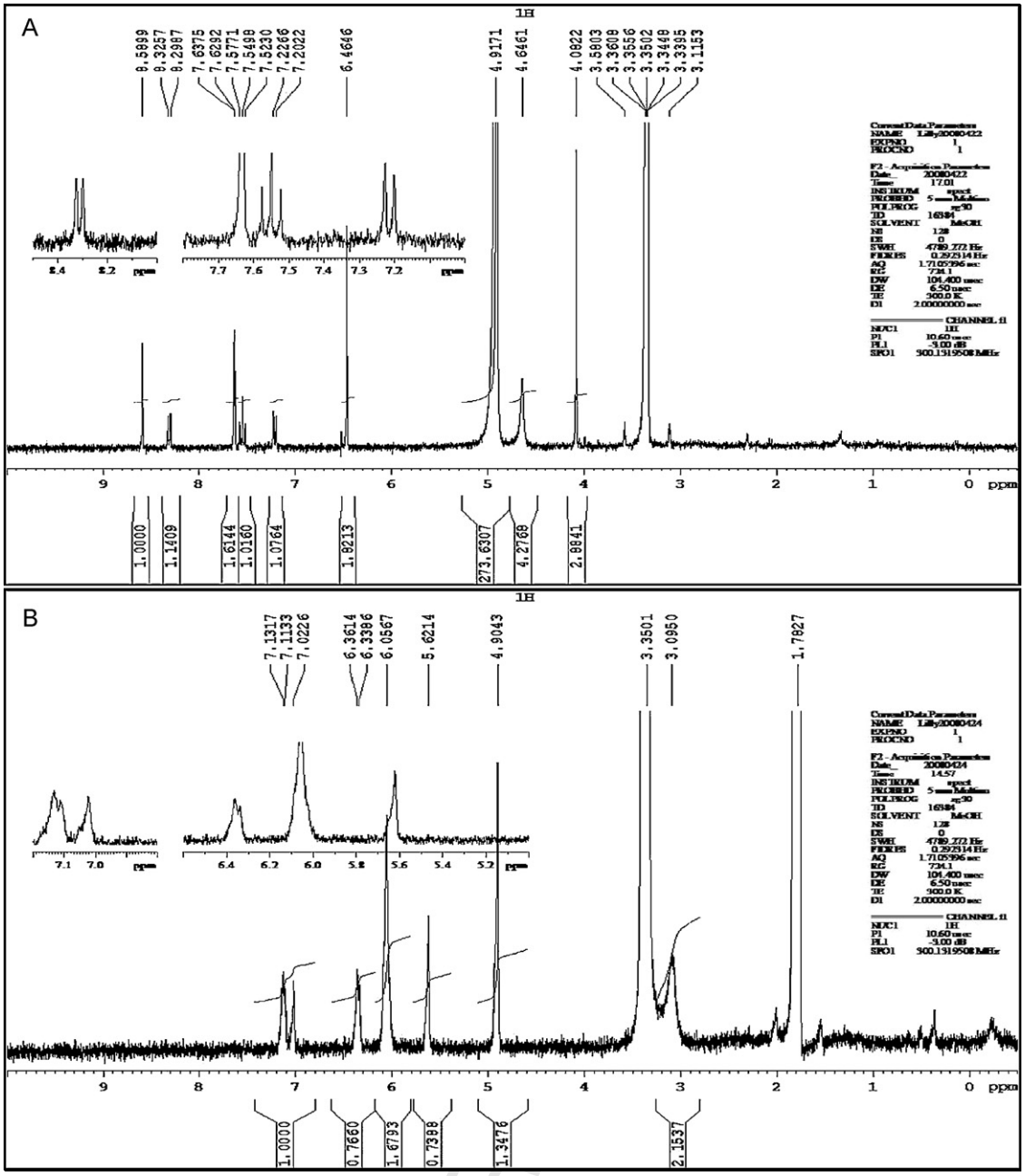

Fig. 3. NMR spectrum of (A) aristolactam I and (B) aristolactam II.

(m/z 294.0) and AL-II (m/z 263.9) are the most abundant, and their product-ion spectra show the same patterns as previously reported (Fig. 2) [8,10]. Structural characterization with NMR, the spectra of ALs in d_4-methanol were shown in Fig. 3 and consistent with previous studies [7,37]. These data provided structural information for specific quantitation of AL-I and AL-II by monitoring the ion pairs m/z 294 → 279 and 264 → 206 to achieve the maximum sensitivity.

3.2. On-line SPE-LC/MS/MS analysis and method validation

Urine is a complex matrix and could interfere the desirable signals, enhance, or suppress the efficiency of ionization of analytes so that sensitivity and specificity of the analytical method could be significantly affected. In this study, on-line SPE was adopted to save labors and time in sample cleanup. The recovery of on-line SPE is approximate to 98.0% for AL-I and 99.5% for AL-II in average (Table 1) and suggests insignificant loss of samples in on-line SPE cleanup. This an advantage of on-line SPE to automate sample cleanup procedures could improve the variability among samples. But Table 1 summarizes the performance of this analytical method. This method demonstrated excellent recovery, stability, and reproducibility in analysis of ALs in urine samples. The limits of detection (LODs) were ranged from 0.3 ng/ml to 1.2 ng/ml at a signal-to-noise ratio of 3. The limits of quantitation (LOQs) were ranged from 0.9 ng/ml to 3.9 ng/ml at a signal-to-noise ratio of 10 corresponding to 0.006 ng and 0.024 ng on column for AL-I and AL-II, respectively (Table 1). The sensitivity of this method was comparable with that of other methods [8,9,32].

This study was to quantify urinary ALs to serve as biomarkers for AAs intake, particularly at very low levels. The matrix effect could

Please cite this article in press as: S.-Y. Chiang, et al., J. Chromatogr. B (2011), doi:10.1016/j.jchromb.2011.06.045

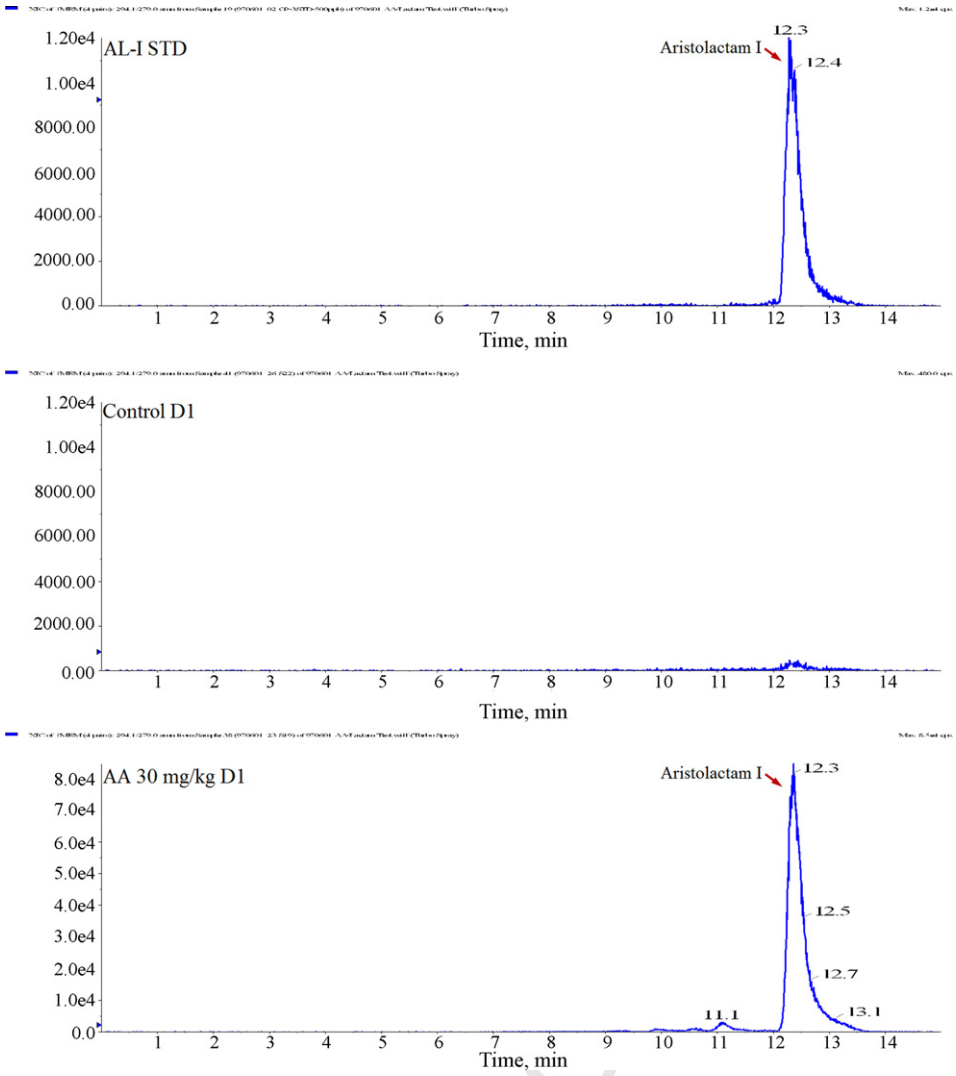

Fig. 4. Representative chromatograms generated from analysis of aristolactam I in urine spiked with aristolactam I standard (AL-I STD) and collected from control mice (Control) and mice treated with AAs on the first day (AA 30 mg/kg D1) with LC/MS/MS operated under MRM mode.

have a great impact on quantitation of urinary ALs. To estimate the influence of the matrix, calibration curve standard solutions from 1.0 to 5000 ng/ml were separately prepared in solvent (50% ACN) and urine. The calibration curves showed excellent linearity for ALs (AL-I: slope: 789.64, R^2 = 0.996 in solvent; slope: 570.64, R^2 = 0.996 in urine; AL-II: slope: 63.21, R^2 = 1.00 in solvent; slope: 48.7, R^2 = 0.9999 in urine). The matrix effects of AL-I and AL-II in urine were calculated by following the equations proposed by Matuszewski et al. [38–40], determined by the ratio of the slopes of the calibration curves in urine divided by those in solvent, and were 75.3 ± 4.5% and 75.4 ± 6.2%, respectively. This method has high recovery and significant matrix effect, and this observation suggests that residues in urine may suppress ionization efficiency for ALs [40]. This may be attributed to a compromise between the use of the on-line SPE and suppression ionization efficiency. Further studies will be needed to elucidate the factors associated with the significant matrix effects in analysis of urinary ALs and to improve the sample cleanup procedures to remove more endogenous compounds to reduce the matrix effects with acceptable recovery [40]. Therefore, the calibration curves prepared by analysis of ALs in urine were used for quantitation of both analytes in this study to reduce the influence of matrix.

3.3. Animal study

In this study, male C3H/He mice were treated with a single dose of 30 mg/kg or 50 mg/kg of AAs. There was no statistical difference between groups in body weight on days 3 and 6, except in mice treated with 50 mg/kg of AAs, which were significantly decreased on day 6 (18.7 ± 0.8 g versus 22.5 ± 2.8 g). The levels of urinary total protein/mg creatinine (Cr) in mice treated with either 30 or 50 mg/kg of AAs were increased on day 2 and reached a plateau on day 4, about 6-fold and 10-fold higher than those of control mice, respectively. After analysis of urine sample, Figs. 4 and 5 show the representative LC/MS/MS chromatograms generated from analysis of urinary AL-I and AL-II in a urine sample collected from either control or treated animal. Three peaks appear on the chromatograms of AL-II for samples originated from AAs-treated mice, but that of the control sample shows no additional peak and no interference from the urine matrix. The AL-II peak was identified and confirmed with the retention time by analysis AL-II standard. The two additional peaks with retention time at 9.9 and 11.0 min could be associated with the treatment of AAs (Fig. 5) and could not be identified since we did not have all the synthesized metabolite standards of AAs. But some MS/MS spectra of 3 hydroxylated aristolactams show that

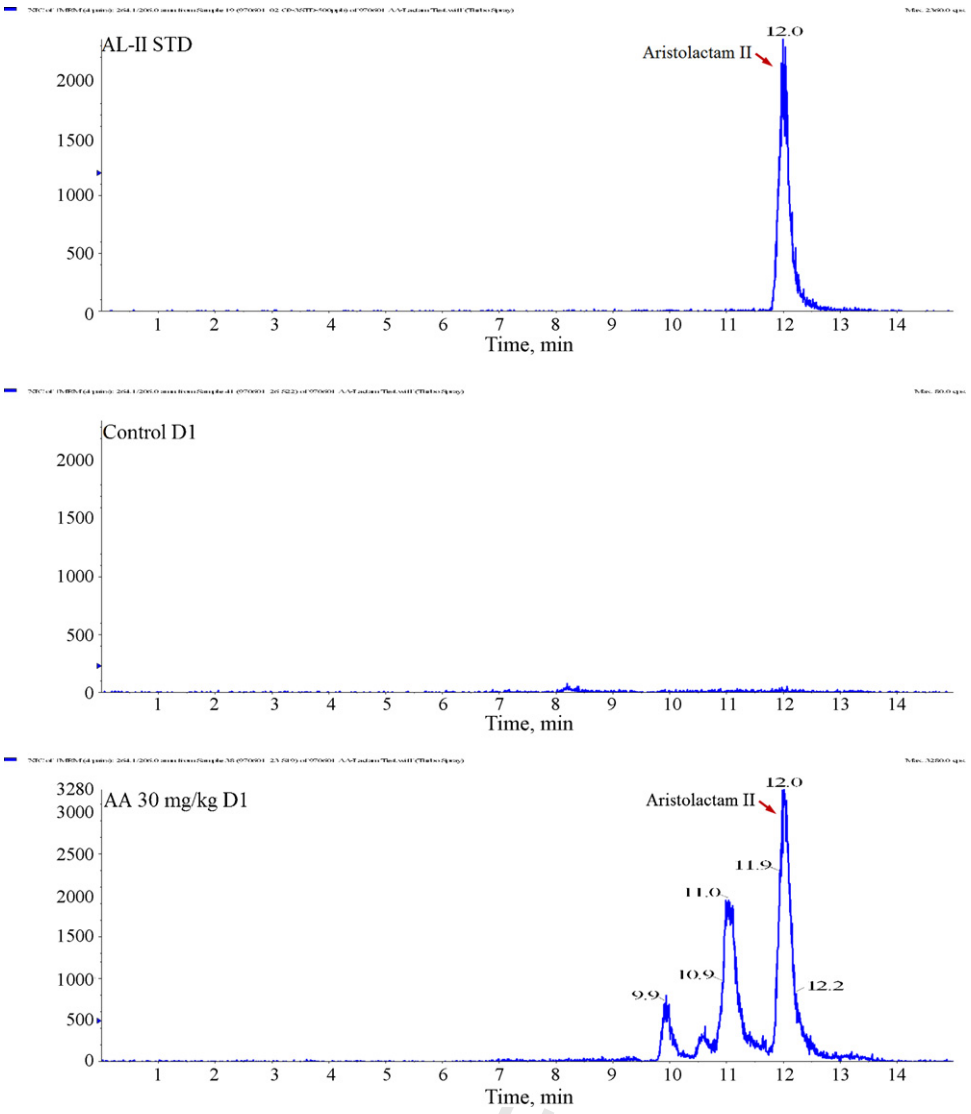

Fig. 5. Representative chromatograms generated from analysis of aristolactam II in urine spiked with aristolactam II standard (AL-II STD) and collected from control mice (Control) and mice treated with AAs on the first day (AA 30 mg/kg D1) with LC/MS/MS operated under MRM mode.

they produced fragment ions at m/z 206 with retention times earlier than AL-II and suggest that these additional peaks might be contributed by N-hydroxyaristolactam, 7-hydroxyaristolactam, or aristolactam 1a [8,9]. One of the advantages in analyzing urinary ALs with tandem mass spectrometry operated under MRM mode was to select the precursor ion for further induced-collision fragmentation so that monitoring the ion pairs of the precursor ions at m/z 294 and 264 and its corresponding product ion at m/z 279 and 206 provides excellent specificity in quantitation.

Our data show that treatment of a single dose of 30 mg/kg of AAs led to excretion of urinary AL-I and AL-II on day 1 at 88.8 ± 36.7 μg/mg Cr and 24.0 ± 5.9 μg/mg Cr, respectively. After a single dose of 50 mg/kg of AAs, the urinary AL-I and AL-II on day 1 were 105.4 ± 35.6 μg/mg Cr and 30.8 ± 9.3 μg/mg Cr, respectively (Table 2). The concentrations of excreted ALs in mouse urine were highest on day 1 ($p < 0.05$), and rapidly decreased to less than 1.65 μg/mg Cr on day 2 and further deceased on day 4 and day 7 (Table 2). The rapid elimination of urinary ALs indicated that ALs have short half-lives. The estimated half-lives were 3.55 h for AL-I and 4.04 h for AL-II (at 30 mg/kg of AAs) and 4.00 h for AL-I and 4.83 h for AL-II (at 50 mg/kg of AAs). However, increases in excretion of urinary protein were observed among the AAs-treated mice and suggested that the kidney function of the treated animals could have been damaged. Further study is needed to investigate the impacts on the kinetics of urinary ALs by the damage of kidney function by AAs treatment. The on-line SPE-LC/MS/MS method will be very helpful if such study will be conducted. According to the results of this study, urinary ALs appears to be suitable biomarkers to assess the current exposure to AAs.

Although urine samples at the first 24 h were collected from rats treated with AA and processed with SPE for analysis of ALs and identification of other AAs metabolites with LC/MS/MS in previous studies [8,9], their objectives were to identify potential metabolites of AAs with mass spectrometry. Sensitivity of the analytical method was not an issue, but characterization of metabolites with LC/MS/MS was very critical [8,9]. This study was to validate urinary ALs to serve as exposure biomarkers for AAs intakes. Therefore, sensitivity of the analytical method was a critical issue after the NMR and mass spectrometry spectra of newly synthesized ALs standards were confirmed to be consistent with previous studies [7–9,37]. In order to correct the matrix effects, quantitation of urinary ALs should be based on the calibration curves established by analysis of ALs standards in urine matrix. This study is the first attempt to develop an analytical method to quantify urinary ALs at extremely

low levels. With the performance of our newly developed method, there may be a good chance to analyze ALs in urine samples collected from people consuming low amounts of AAs from herbal medicine. Therefore, this on-line SPE-LC/MS/MS method may definitely help epidemiologists who have been interested in molecular epidemiology studies on the potential effects of low intake of AAs.

4. Conclusions

An on-line SPE-LC/MS/MS method was successfully developed to analyze the most abundant metabolites, ALs, of AAs in urine. Our results demonstrated that this method possesses excellent sensitivity and specificity compared with previously reported methods. Moreover, this method simplified the usually labor- and time-consuming sample pretreatment procedures. The *in vivo* study showed that urinary ALs were rapidly excreted after AA treatment and suggests that analysis of urinary ALs may serve as AA exposure biomarkers. By using this method, urinary ALs can be analyzed rapidly and easily to serve as biomarkers of current intake of AAs from Chinese herbs.

Q2 Uncited reference

[5].

Acknowledgements

The authors are very thankful to Li-Ching Shen for her excellent technical assistance. This study was supported in part by research grants from the National Science Council of Taiwan (NSC93-2320-B-039-049) and China Medical University (CMU95-051).

References

[1] S.C. Hsieh, M.F. Huang, B.S. Lin, H.T. Chang, J. Chromatogr. A 1105 (2006) 127.
[2] V.M. Arlt, M. Stilborova, H.H. Schmeiser, Mutagenesis 17 (2002) 265.
[3] F.D. Debelle, J.L. Vanherweghem, J.L. Nortier, Kidney Int. 74 (2008) 158.
[4] Y.Y. Chen, S.Y. Chiang, H.C. Wu, S.T. Kao, C.Y. Hsiang, T.Y. Ho, J.G. Lin, Acta Pharmacol. Sin. 31 (2010) 227.
[5] V.M. Arlt, M. Stiborova, J. vom Brocke, M.L. Simoes, G.M. Lord, J.L. Nortier, M. Hollstein, D.H. Phillips, H.H. Schmeiser, Carcinogenesis 28 (2007) 2253.
[6] G. Krumbiegel, J. Hallensleben, W.H. Mennicke, N. Rittmann, H.J. Roth, Xenobiotica 17 (1987) 981.
[7] W. Pfau, H.H. Schmeiser, M. Wiessler, Carcinogenesis 11 (1990) 313.
[8] W. Chan, L. Cui, G. Xu, Z. Cai, Rapid Commun. Mass Spectrom. 20 (2006) 1755.
[9] W. Chan, H.B. Luo, Y. Zheng, Y.K. Cheng, Z. Cai, Drug Metab. Dispos. 35 (2007) 866.
[10] W. Chan, Y. Zheng, Z. Cai, J. Am. Soc. Mass Spectrom. 18 (2007) 642.
[11] K. Hashimoto, M. Higuchi, B. Makino, I. Sakakibara, M. Kubo, Y. Komatsu, M. Maruno, M. Okada, J. Ethnopharmacol. 64 (1999) 185.
[12] J.L. Vanherweghem, Lancet 349 (1997) 1399.
[13] M. Zhu, J. Phillipson, Pharm. Biol. 34 (1996) 283.
[14] T. Lee, M. Wu, J. Deng, D. Hwang, J. Chromatogr. B 766 (2001) 169.
[15] E.S. Ong, S.O. Woo, Y.L. Yong, J. Chromatogr. A 904 (2000) 57.
[16] J. Yuan, L. Nie, D. Zeng, X. Luo, F. Tang, L. Ding, Q. Liu, M. Guo, S. Yao, Talanta 73 (2007) 644.
[17] C. Zhang, X. Wang, M. Shang, J. Yu, Y. Xu, Z. Li, L. Lei, X. Li, S. Cai, T. Namba, Biomed. Chromatogr. 20 (2006) 309.
[18] W. Chan, K.C. Lee, N. Liu, Z. Cai, J. Chromatogr. A 1164 (2007) 113.
[19] J. Yuan, Q. Liu, W. Zhu, L. Ding, F. Tang, S. Yao, J. Chromatogr. A 1182 (2008) 85.
[20] W. Li, S. Gong, D. Wen, B. Che, Y. Liao, H. Liu, X. Feng, S. Hu, J. Chromatogr. A 1049 (2004) 211.
[21] S. Shi, W. Li, Y. Liao, Z. Cai, H. Liu, J. Chromatogr. A 1167 (2007) 120.
[22] F. Wei, Y.Q. Feng, Talanta 74 (2008) 619.
[23] S.A. Chan, M.J. Chen, T.Y. Liu, M.R. Fuh, J.F. Deng, M.L. Wu, S.J. Hsieh, Talanta 60 (2003) 679.
[24] G.C. Kite, M.A. Yule, C. Leon, M.S. Simmonds, Rapid Commun. Mass Spectrom. 16 (2002) 585.
[25] M. Lee, C. Tsao, S. Iou, W. Chuang, S. Sheu, J. Sep. Sci. 26 (2003) 818.
[26] J. Yuan, Q. Liu, G. Wei, F. Tang, L. Ding, S. Yao, Rapid Commun. Mass Spectrom. 21 (2007) 2332.
[27] F.Y. Yu, Y.H. Lin, C.C. Su, J. Agric. Food Chem. 54 (2006) 2496.
[28] S.M. Chen, M.Y. Fan, C.C. Tseng, Y. Ho, K.Y. Hsu, Toxicon 50 (2007) 180.
[29] H. Yue, W. Chan, L. Guo, Z. Cai, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 877 (2009) 995.
[30] X. Fu, Y. Liu, W. Li, N. Pang, H. Nie, H. Liu, Z. Cai, Electrophoresis 30 (2009) 1783.
[31] C.H. Kuo, C.W. Lee, S.C. Lin, I.L. Tsai, S.S. Lee, Y.J. Tseng, J.J. Kang, F.C. Peng, W.C. Li, Talanta 80 (2010) 1672.
[32] Z. Ling, Z.Z. Jiang, X. Huang, L.Y. Zhang, X.Y. Xu, Biomed. Chromatogr. 21 (2007) 10.
[33] K. Stoob, H.P. Singer, C.W. Goetz, M. Ruff, S.R. Mueller, J. Chromatogr. A 1097 (2005) 138.
[34] T.Y. Wu, M.R. Fuh, Rapid Commun. Mass Spectrom. 19 (2005) 775.
[35] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, Anal. Bioanal. Chem. 383 (2005) 638.
[36] C.M. Li, C.W. Hu, K.Y. Wu, J. Mass Spectrom. 40 (2005) 511.
[37] B. Achari, S. Bandyopadhyay, A.K. Chakravarty, S.C. Pakrashi, Org. Magn. Reson. 22 (1984) 741.
[38] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[39] W.C. Shih, M.F. Chen, C.C. Huang, S.N. Uang, S.T. Shih, S.H. Liou, K.Y. Wu, Rapid Commun. Mass Spectrom. 21 (2007) 4073.
[40] I. Marchi, V. Viette, F. Badoud, M. Fathi, M. Saugy, S. Rudaz, J.-L. Veuthey, 1217 Q3 (2010) 4071.