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Determination of aristolochic acid in Chinese herbal medicine by capillary electrophoresis with laser-induced fluorescence detection

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Abstract

We have demonstrated the analysis of aristolochic acids (AAs) that are naturally occurring nephrotoxin and carcinogen by capillary electrophoresis in conjunction with laser-induced fluorescence detection (CE-LIF). Owing to lack of intrinsic fluorescence characteristics of oxidized AAs (OAAs), reduction of the analytes by iron powder in 10.0 mM HCl is required prior to CE analysis. The reduced AAs (RAAs) exhibit fluorescence at 477 nm when excited at 405 nm using a solid-state blue laser. By using 50.0 mM sodium tetraborate (pH 9.0) containing 10.0 mM SDS, the determination of AA-I and AA-II by CE-LIF has been achieved within 12 min. The CE-LIF provides the LODs of 8.2 and 5.4 nM for AA-I and AA-II, respectively. The simple CE-LIF method has been validated by the analysis of 61 Chinese herbal samples. Prior to CE analysis, OAAs were extracted by using 5.0 mL MeOH, and then the extracts were subjected to centrifugation at 3000 rpm for 5 min. After reduction, extraction, and centrifugation, the supernatants were collected and subjected to CE analysis. Of the 61 samples, 14 samples contain AA-I and AA-II, as well as 10 samples contain either AAI or AAII. The relative standard deviation (RSD) values of the migration times for AA-I and AA-II are less than 2.5% and 2.1% for three consecutive measurements of each sample. The RSD values for the peak heights corresponding to AA-I and AA-II in most samples are about 8.0% and 10.0%, respectively. The result shows that the present CE-LIF approach is sensitive, simple, efficient, and accurate for the determination of AAs in real samples.
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Keywords: Aristolochic acids; Capillary electrophoresis; Chinese herbal medicine; Laser-induced fluorescence

1. Introduction

Aristolochic acids (AAs), a family of structurally related nitrophenanthrene carboxylic acids, are found naturally in medicinal plants such as Radix aristolochiae or herbs such as *Aristolochia* spp. [1]. The herbs sold on the Asian market are used to relieve pain by subdueing hyperactivity of the liver, to induce diuresis, as well as to control weight [2–4]. In the early 1990s, ingestion of AAs was incriminated in the outbreak of the so-called Chinese herbs nephropathy (CHN), severe tubulointerstitial nephritis [5,6]. In addition to a rapidly progressive interstitial renal failure due to particularly severe fibrosis, the clinical course of CHN is complicated by tumor transformations in the urothelium [7]. Many countries including UK, Canada, Australia, and Germany have announced banning to restrict the importation, sale, and use of AA-containing medicines like *Aristolochia fangchi* and Mutong [8]. In 2001, the Food and Drug Administration (FDA) in USA issued warnings and an important alert that herbal products are unsafe if they contain or are suspected to contain AA [9]. In the November of 2003, five Chinese herbal drugs containing AAs were officially banned in Taiwan. Despite the action of the FDA and the effort of many countries, 19 products containing AAs and 95 products suspected to contain AAs were for sale on the Web in 2003 [10]. Because AAs have threatened the public health, there is still a need to develop simple, sensitive and effective methods to detect the presence of AAs in Chinese herbs remedies and dietary supplements.

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There are many AAs, including two predominant ones that are 8-methoxy-6-nitrophenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (AA-I) and 6-nitro-phenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (AA-II). The analysis of AA-I and AA-II is difficult mainly because of their extremely similar structures and identical medicinal activities. Thin layer chromatography (TLC) [11–13], UV–vis absorption [14] and high performance liquid chromatography (HPLC) with UV–vis absorption detection (HPLC/UV) [15–18] have been applied to the analyses of AA-I and/or AA-II in samples. In order to further confirm the structural identification of compositions of Chinese herbs, HPLC with quadrupole ion-trap mass spectrometry (MS) and with electrospray ion-trap MS detection have been employed [19–21]. Quantitative determinations of AA-I in the incriminated preparations were also demonstrated using both UV–vis absorption and MS detection [22].

Capillary electrophoresis (CE) provides the advantages over HPLC, including high speed, efficiency, and requiring minute amounts of samples and reagents, and has proved to be a powerful tool for the analysis of polar and thermally labile compounds [23]. Analyses of natural medicines and natural products by CE have been well documented, especially in Chinese Society [24–26]. CE with UV–vis absorption detection has been developed for the analysis of AA-I and AA-II in Chinese prepared medicines (CPMs) after pressurized liquid extraction [27,28]. The limits of detection (LODs) at a signal-to-noise ratio (S/N) = 3 for AA-I and AA-II were found to be 1.2 and 0.9 mg/L (3.5 and 2.9 mM), respectively. Because of poor sensitivity and less selectivity, the method required great amounts of samples and reagents, as well as tedious sample preparations. In addition, most solutes in CPMs absorb UV light, which cause analysis errors when co-migration of the analytes takes place.

The aim of this work was to develop a simple, rapid, and sensitive method for the determinations of AA-I and AA-II in medicinal samples and dietary supplements by CE. In order to provide great sensitivity, laser-induced fluorescence (LIF) detection was employed in the CE system. Because AAs have weak/no fluorescence, their nitro groups have to be reduced to amino groups in order to achieve high sensitivity of CE-LIF. The reduction of the nitro group was performed in acidic solutions containing iron powder [29,30]. We carefully evaluated parameters such as organic additives, surfactants, pH, and ionic strength that affect the separation selectivity and speed, as well as detection sensitivity of the method. The proposed method was also validated by the analyses of 61 medicinal samples and dietary supplements.

2. Materials and methods

2.1. Standard chemicals

AAs, sodium tetraborate, and iron powder (325-mesh) were purchased from Acros organics (New Jersey, USA). The AA standard contains 90% AA-I and 10% AA-II. Methanol of analytical reagent grade was obtained from Mallinckrodt (Phillipsburg, NJ, USA). Sodium dodecyl sulfate (SDS) was obtained from Sigma (St. Louis, MO, USA). Sodium hydroxide and hydrochloric acid were obtained from Riedel-de Haën (Seelze, Germany). Ethylenediaminetetraacetic acid (EDTA) was purchased from Kanto Chemical (Tokyo, Japan). Sodium tetraborate solutions (10.0–70.0 mM) were adjusted with 1.0 M HCl or 0.5 M NaOH to the desired pH values (4.0–9.0).

2.2. Medicinal samples

The medicinal samples and dietary supplements used in this study were either obtained from patients or purchased from local drug stores. Patients who have taken these herbs medicines or dietary supplements for some times are suspected to be the case of CHN in the Shin Kong Wu Ho-Su Memorial Hospital (Taipei, Taiwan). Most of the herb medicines or dietary supplements are powder, and some are either pills or capsules. The samples in a pill formulation were ground into fine powder before conducting sample preparation. The sugar shells of capsules were removed and the fine powders were used for further sample preparation.

2.3. Sample preparation

Approximately 0.20 g of the medicinal powders and dietary supplements were dissolved in aliquots of 5.0 mL of MeOH. In order to ensure completed dissolution of the powders in methanol, the solutions were subjected to ultrasonication at room temperature for 30 min. The solutions were then subjected to centrifugation at 3000 rpm for 5 min and the supernatants were carefully collected by pipetting.

2.4. Reduction of AA-I and AA-II

For simplicity, hereafter the oxidized AA-I and AA-II are denoted by OAA-I and OAA-II, while their reduced forms are denoted by RAA-I and RAA-II, respectively. The stock OAA solution was prepared by dissolving 2.0 mg standard mixture of OAA-I (90%) and OAA-II (10%) in methanol into a 10.0 mL volumetric flask. The reductions of OAA-I and OAA-II were conducted by adding 173.0 μL of the stock OAA solution to 10.0 mL of 10.0 mM HCl solution containing 10.0 mg iron powder. Under this condition, most iron powder was not dissolved. The concentrations of OAA-I and OAA-II in thus-prepared solution are 9.0×10^{-6} M and 10^{-6} M, respectively. The solutions were stirred by using a magnetic stirrer at room temperature for 40 min unless otherwise noted, during which time the solution changed color from pale yellow to colorless. When conducting reduction of OAAs from the real samples, instead of 173.0 μL of the stock AA solution, aliquots of 1.0 mL of the super-

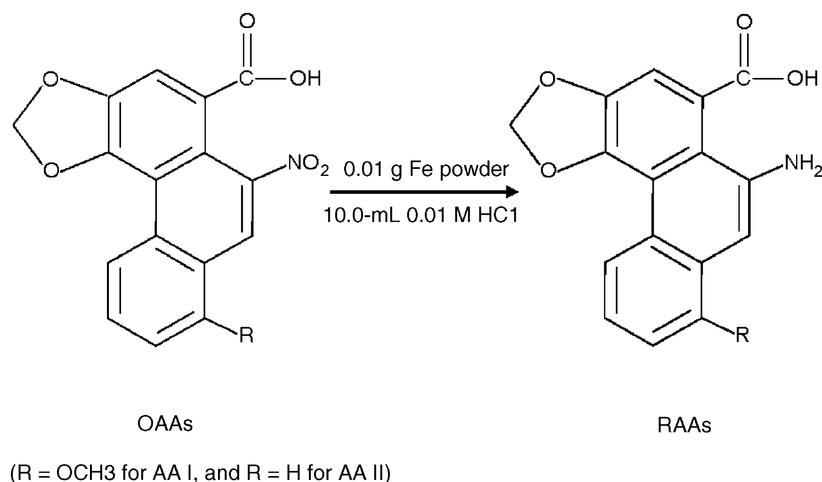

Fig. 1. Reduction of OAAs to RAAs by 0.01 g iron powder in 10.0 mM HCl. R separately represents OCH_3 and H in OAA-I and OAA-II.

natants were separately added to vials containing 9.0 mL of 10.0 mM HCl solution and 10.0 mg iron powder. Fig. 1 exhibits the reaction of OAAs to RAAs. For fluorescence, IR, MS, ^{1}H NMR, and UV–vis absorption measurements, 5.0 mL of the stock AA solution (0.2 mg/mL) was added to 95.0 mL of 10.0 mM HCl solution containing 0.1 g iron powder. After the reduction was completed, RAAs were extracted from aqueous solution to CH_2Cl_2. To obtain RAA powder, the CH_2Cl_2 layer was collected and dried by using a rotary evaporator at 30 °C. The powder was then re-dissolved in buffers or in organic solvent (CD_3OD), or mixed with KBr (details see Section 2.5).

2.5. Characterization

A double-beam UV–vis spectrophotometer (Cintra 10e, GBC Scientific Equipment Pty Ltd., Dandenong, Victoria, Australia) was used to measure the absorption values of 10^{-5} M OAAs and RAAs solutions. Fluorescence spectra were recorded using a fluorometer (Aminco–Bowman Series 2, ThermoSpectronic, Pitsford, NY, USA). Prior to UV–vis absorption and fluorescence measurements, the OAAs and RAAs powders were separately dissolved in 3.0 mL methanol, and then diluted to 1/50 with 50.0 mM sodium tetraborate (pH 2.0–9.0). An IR spectrophotometer (MAGAN-IR 550, Nicolet, New York, NY, USA) was used to collect the IR spectra of the OAAs and RAAs. The powders were separately mixed with KBr powder by a ratio of 1:5 (w/w) and then the mixtures were platted to a thin pill prior to IR measurements. A FAB MS (JOEL SX-102A, JOEL, Peabody, MA, USA) was used to measure the mass spectra of OAAs and RAAs. The samples were prepared by dissolving OAAs and RAAs powder in nitrobenzene alcohol matrix. For collecting the ^{1}H NMR spectra using a Varian 400 MHz NMR (Palo Alto, CA, US), 1.0 mg OAA and RAA powder were separately dissolved in 1-mL CD_3OD.

2.6. CE-LIF apparatus

The basic design of the separation system has been previously described [31]. Briefly, a high-voltage power supply (Gamma High Voltage Research Inc., Ormond Beach, FL, USA) was used to drive electrophoresis. The entire detection system was enclosed in a black box with a high-voltage interlock. The high-voltage end of the separation system was housed in a plexiglass box for safety. A 4.0-mW solid state laser with 405 nm output from B&WTEK Inc. (Newark, DE) was used for excitation. The emission light was collected with a 10× objective (numeric aperture = 0.25). One RG 450 cutoff filter was used to block scattered light before the emitted light reached the phototube (R928, Hamamatsu Photonics K. K., Shizuoka-Ken, Japan). The fluorescence signal was transferred directly through a 10-kΩ resistor to a 24-bit A/D interface (CSW chromatographic station; DataApex Ltd., Czech Republic) at 10 Hz and stored in a PC. Capillaries with 75 μm I.D. and 365 μm O.D. were from Polymicro Technologies (Phoenix, AZ, USA).

2.7. CE conditions and quantitative analysis

The 40-cm capillary was rinsed with 0.5 N NaOH overnight prior to use for electrophoresis. The RAA sample was injected into a capillary filled with 10–70.0 mM sodium tetraborate containing 0–30.0 mM SDS (pH 9.0) at 20-cm height for 10 s. The separation was conducted at 10 kV. Between consecutive runs, the capillary was flushed with the running buffer for 1 min to guarantee good reproducibility. In order to determine the concentrations of the analytes, 100.0 μL of RAA_S standard solutions (10^{-5} to 5×10^{-7} M) were spiked into 900.0 μL of sample solutions. Linear plots of the peak heights against the concentrations of the standards were depicted and the plots were used to determine the concentrations of the analytes in the samples.

3. Results

3.1. Reduction of OAAs and characterization

Oxidative enzymes such as hepatic microsomal CYP1A1/2, NADPH:CYP reductase, DT-diaphorase and cyclooxydase-1 cause reduction of the nitro group in AA-I and AA-II to form reactive cyclic nitrenium ions [32,33]. In the presence of iron powder at pH 2.0, we anticipated that the nitro groups in OAA-I and OAA-II were reduced to amino groups and thus RAA-I and RAA-II formed, respectively, as shown in Fig. 1. In order to identify the products, IR, NMR, and MS measurements were performed. Table 1 lists some of the IR, NMR, and MS data for OAAs and RAAs. The disappearance of NO_2 signals in IR (1347, 1525, and 1594 cm^{-1}) and MS ($m/z = 341$ and 312) as well as the appearance of NH_2 signals in the spectra of IR (1635 and 3220 cm^{-1}), NMR ($\delta = 0.87$ and 5.30), and MS ($m/z = 311$ and 282) strongly support the formation of RAAs. Differential UV–vis absorption and fluorescence characteristics between OAAs and RAAs further support our reasoning.

Fig. 2A clearly exhibits that OAAs and RAAs possess differential UV–vis absorption spectra. The absorption bands of OAAs at 219, 251 and 316 nm correspond to the carboxyl group, conjugated olefin group (aromatic compounds), and nitro group, respectively. A broad absorption band between 225 and 288 nm for the RAAs further suggests the formation of aniline compounds [34]. Although the absorbance values at 316 nm for the OAAs and RAAs are close, there is no apparent band at 316 nm in RAAs as that in OAAs. Fig. 2B

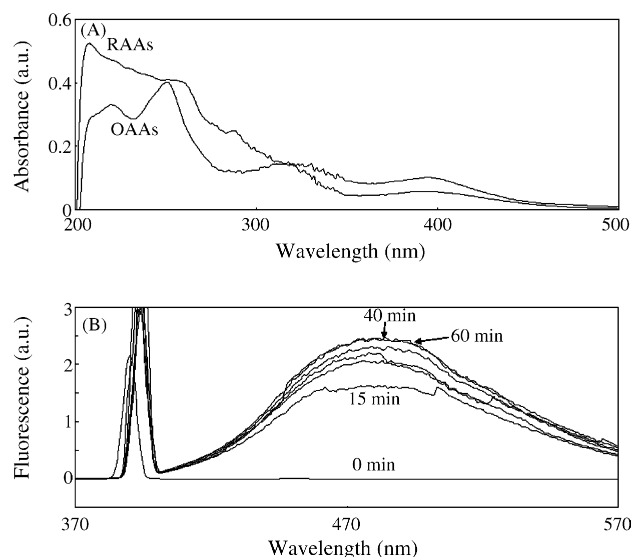

Fig. 2. (A) UV–vis spectra of OAAs and RAAs in 50.0 mM sodium tetraborate solution at pH 9.0. (B) Time evolution of fluorescence spectra of RAAs. The excitation wavelength is 390 nm and the emission wavelength is 477 nm.

Table 1
Comparisons of OAAs and RAAs with respect to IR, ^{1}H NMR, Mass, UV–vis absorption, and fluorescence data

Spectrochemical methods	OAAs	RAAs
IR (wavelength, cm^{-1})	1270 (R—O—CH_3) 1347 (R—NO_2) 1525 (R—NO_2) 1642 (aromatic ring) 1659 (aromatic ring) 1594 (R—NO_2) 1697 (C=O stretching)	1044 (R—O—CH_3) 1270 (R—O—CH_3) 1607 (aromatic ring) 1642 (aromatic ring) 1659 (aromatic ring) 1635 (N—H bending) 3220 (N—H stretching)
^{1}H NMR (ppm)	1.23 (saturated —CH_3) 1.60 (saturated –CH) 2.15 (Ar—CH) 4.04 (—OMe) 6.37 (Ar—H) 7.24 (Ar—H)	0.87 (R_2—NH) 1.23 (saturated —CH_3) 1.60 (saturated —CH) 1.86 (saturated —CH) 1.99 (saturated —CH) 4.01 (—OMe) 4.04 (—OMe) 5.30 (Ar—NH_2) 6.37 (Ar—H) 7.24 (Ar—H)
Mass (m/z)[a]	312 $[M1 + H—OCH_2]^+$, $[M2 + H]^+$ 341 $[M1]^{+}$[b]	282 $[M1 + H—OCH_2]^+$, $[M2 + H]^+$ 311 $[M1]^{+}$[b]
UV–vis absorption (nm)	219 (—COOH) 251 (Triolefin, aromatic ring) 316 (—NO_2) 395	225 (Ar—NH_2) 259 288 (Ar—NH_2) 395
Fluorescence (nm)	None	$\lambda_{ex} = 390$ nm; $\lambda_{em} = 477$ nm

[a] $M1$ and $M2$ represent the formular weights of AA-I and AA-II, respectively.
[b] The peaks for OAAs and RAAs are not shown as the base peaks.

exhibits that OAAs (pH 2.0) do not fluoresce at 477 nm when excited at 390 nm, but the solution fluoresced in the presence of iron powder. The fluorescence reached a plateau at the reaction time of 40 min, suggesting that the formation of RAAs from the reduction of OAAs was completed within 40 min. The reason that OAAs do not fluoresce is mainly because of quenching caused by NO_2 group (withdrawing group). Increases in fluorescence with increasing pH in the range of 2.0–9.0 suggest that an intra hydrogen bond formed between the amino group and carboxylate in RAAs. As a result of formation of a rig structure, the RAAs molecules rotated to a less extent and thus fluoresced more strongly. The fluorescence intensity at pH 8.0 was about 1.6 times higher than that at pH 2.0. Because there were Fe^{3+} ions in the solution, it is possible that the increase in fluorescence was due to the formation of RAAs-Fe(III) complexes. To test the possibility, we added EDTA that forms a strong complex with Fe^{3+}at the concentration ranges 10^{-2} to 10^{-6} M to the RAAs solution (pH 9.0). The fact of slight changes in the fluorescence intensity in the presence of EDTA rules out the possibility of forming RAAs-Fe(III) complexes.

3.2. Optimization of CE separation and LIF detection

In addition to low quantum yields at low pH values (<4.0), RAA-I and RAA-II were not separated in the baseline, mainly because they have similar structures and their amino groups are both fully protonated. Thus we focused on evaluating parameters that affect the separation speed, resolution, sensitivity, and reproducibility at the pH values ranging from 4.0 to 9.0, including compositions, pH, and ionic strength of the background electrolytes, additives (SDS), and applied voltage.

To minimize the quenching caused by Joule heats and the loss of resolution due to analyte adsorption, selection of a suitable background electrolyte in CE-LIF is extremely important. On the basis of our previous study [35], we have learned that sodium tetraborate buffer provides higher separation efficiency and sensitivity for amines than do phosphate or carbonate buffer solution. We thus used 50 mM tetraborate solutions to test the pH dependence of the separation resolution and reproducibility of RAAs in CE-LIF. In the pH range of 4.0–9.0, we found that the separation is proper at pH 9.0 mainly because the EOF is constant as well as minimum analyte adsorption. At pH value above 7.0, the analyes migrated with high electrophoretic mobilities against high EOF. The instability of RAAs and high Joule heats generated that caused fluorescence quenching and irreproducibility are problematic at pH > 9.0.

Next we tested the ionic strength dependence of the sensitivity and resolution of RAA-I and RAA-II at pH 9.0. The electrophoretic mobilities of RAA-I and RAA-II decreased with increasing ionic strength in the concentration range of 10.0–70.0 mM tetraborate solutions (pH 9.0). The resolution values between RAA-I and RAA-II in 10.0, 30.0, 50.0, and 70.0 mM tetraborate solutions were 1.5, 1.7, 2.1, and 0, respectively. One other drawback of using 70 mM tetraborate solutions is a significant quenching of the fluorescence signals of RAAs, mainly due to Joule heats (collision). Our reasoning is supported by the fact that the currents were 15.0 and 190.0 μA in 10.0 and 70.0 mM tetraborate solutions (pH 9.0), respectively. Although quenching and Joule heats increased with increasing tetraborate concentration, the efficiency was greater in 50 mM tetraborate solution as a result of smaller analyte adsorption. Capillaries with small sizes such as 50 and 25 μm in diameter can be used to reduce the effect of Joule heating, but more difficulty of alignment, problems of analyte adsorption, and loss of sensitivity must be evaluated.

Although 50 mM tetraborate buffer (pH 9.0) provides the baseline separation between RAA-I and RAA-II within 8 min, the peak widths of the RAAs were both wider than 0.4 min. In order to decrease the peak width and thus improve efficiency, SDS was added to the background electrolyte. Table 2 lists the effect of SDS on speed, resolution, sensitivity, and reproducibility for the separation of RAA-I and RAA-II, exhibiting that 10.0 mM SDS is proper. We note that a high fluorescence background and quenching caused by Joule heats are problematic at high SDS concentrations (>20.0 mM). Fig. 3 presents the separation of standard RAA-I and RAA-II at 10 kV using 50.0 mM tetraborate buffer (pH 9.0) containing 10.0 mM SDS. At the separation condition, SDS molecules (critical micelle concentration 8.3 mM) formed micelles, leading to longer migration times for the RAAs because they participated in aqueous solution and micelles. The increase in the separation time was partially

Table 2
Effect of SDS on speed, resolution, sensitivity, and reproducibility for RAA-I and RAA-II by CE-LIF[a]

SDS (mM)	Migration time (min) (RSD)[b]		Resolution (R_s)[c]	LOD (10^{-8} M) (RSD)[b]	
	RAA-II	RAA-I	RAA-II/RAA-I	RAA-II	RAA-I
0	7.51 (0.6%)	8.35 (0.8%)	2.1	2.05 (3.9%)	2.79 (4.2%)
5	8.16 (0.6%)	8.85 (0.7%)	2.4	1.32 (4.1%)	1.69 (4.4%)
10	9.42 (0.5%)	10.28 (0.7%)	3.1	0.54 (4.5%)	0.82 (5.3%)
20	14.64 (1.1%)	15.18 (1.2%)	1.6	0.72 (4.9%)	1.05 (5.9%)
30	21.22 (3.1%)	21.58 (3.5%)	0.6	1.48 (5.8%)	2.62 (7.1%)

[a] Other experimental conditions are the same as in Fig. 3.
[b] $n = 3$.
[c] $R_s = 2(t_1 - t_2)/(W_1 + W_2)$, where t_1, t_2 are the migration times for RAA-I and RAA-II, and W_1, W_2 are the peak widths for RAA-I and RAA-II, respectively.

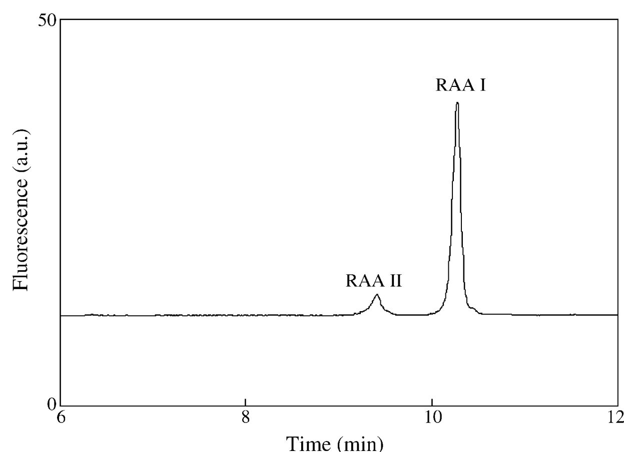

Fig. 3. Electropherogram of the separation of RAA-I and RAA-II by CE-LIF at 10 kV. Capillary: 40 cm in total length, and 30 cm in effective length; filled with 50.0 mM sodium tetraborate (pH 9.0) containing 10.0 mM SDS. The hydrodynamic injection was conducted at 20-cm height for 10 s. The RAA-I and RAA-II were reduced from OAA-I and OAA-II at the concentrations of 9.0×10^{-7} M and 10^{-7} M, respectively.

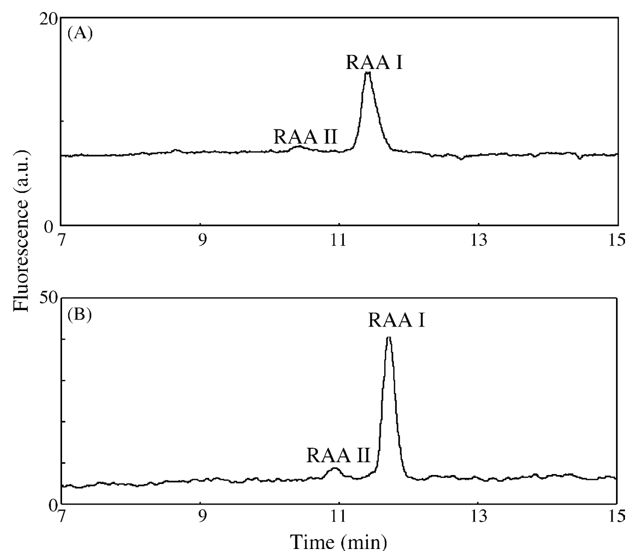

Fig. 4. Electropherograms for the separations of real samples: (A) and (B) samples are numbered 7 and 16 as listed in Table 3. Other conditions are the same as in Fig. 3.

due to increases in the ionic strength. The peak widths for RAA-I and RAA-II were 0.32 and 0.24 min, respectively. As a result, a greater resolving power of 3.1 was obtained. The RSD values for the migration times of RAA-I and RAA-II were 0.5–0.7%, respectively, while the RSD values of their corresponding peak heights were 5.3% and 4.5%, respectively. The LODs at a S/N = 3 for AA-I and AA-II were found to be 8.2 and 5.4 nM, respectively, when injected at 20-cm height for 10 s (the electropherogram as shown in Fig. 3). The sensitivity is about two orders of magnitude higher than that obtained by CE with UV–vis absorption detection [27]. The result suggests that the present method is suitable for the analysis of AAs in medicinal plants and herbs.

3.3. Analysis of medicinal samples and dietary supplements

With a good resolving power and high sensitivity, the present CE-LIF approach was applied to the determination of AAs prepared from small amounts of samples. The AAs were prepared from 0.2 g powder samples, which are less than those (1.0–5.0 g) used in other methods [22,25,27]. We analyzed 61 medicinal samples and dietary supplements, in which 28 samples were sold from drugstores and 33 samples were from patients. Fig. 4 shows two representative electropherograms for the samples numbered 7 and 16 that are listed in Table 3. Because a UV-laser was employed in the CE-LIF system, only solutes with intrinsic fluorescence characteristics can be detected. As a result, there are only few peaks in the two electropherograms; minimizing interferences. The RSD values ($n = 3$) of the migration time for RAA-I and RAA-II were less than 1.2% and 1.0% for the two samples. By applying a standard addition method, the concentrations of OAAs in the samples were determined and the results were

Table 3
Determinations of AA-I and AA-II in medicinal samples and dietary supplements

Samples	Product presentation	AA-I (ppm)[a]	AA-II (ppm)[a]
From drugstores			
1	Powder	5.0 ± 1.0	16.0 ± 2.0
2	Powder	8.0 ± 1.0	–[b]
3	Powder	40.0 ± 3.0	2.0 ± 0.1
4	Tea bags	–	3.0 ± 0.2
5	Powder	120.0 ± 8.0	10.0 ± 1.0
6	Pills	10.0 ± 1.0	1.0 ± 0.1
7	Powder	40.0 ± 3.0	3.0 ± 0.4
8	Powder	15.0 ± 3.0	2.0 ± 0.1
From patients[c]			
9	Powder	20.0 ± 2.0	–
10	Powder	8.0 ± 1.0	–
11	Powder	20.0 ± 2.0	–
12	Powder	50.0 ± 4.0	–
13	Powder	65.0 ± 5.0	8.0 ± 1.0
14	Pills	85.0 ± 6.0	10.0 ± 1.0
15	Powder	5.0 ± 1.0	15.0 ± 2.0
16	Pills	150.0 ± 10.0	12.0 ± 1.0
17	Pills	45.0 ± 4.0	5.0 ± 1.0
18	Pills	5.0 ± 1.0	–
19	Powder	5.0 ± 1.0	3.0 ± 0.3
20	Powder	32.0 ± 3.0	4.0 ± 0.3
21	Powder	10.0 ± 2.0	–
22	Powder	–	8.0 ± 1.0
23	Pills	40.0 ± 3.0	–
24	Pills	100.0 ± 8.0	10.0 ± 1.0

[a] $n = 3$.
[b] Were not detected.
[c] The medicine were obtained from patients who have taken the medicines for some time and have been diagnosed to have renal failure in the Shin Kong Wu Ho-Su Memorial Hospital. Samples 1–2 were from the same pharmaceutical factory. A series of samples from 9 to 12, 13 to 15, 16 to 18 were colleted from different patients, respectively.

listed in Table 3. Of the 61 samples, 14 samples contained AA-I and AA-II, as well as 10 samples contained either AA-I or AA-II. The results of the sample containing AAs are listed in Table 3, with the concentrations ranging from 1.0 to 150.0 ppm. The RSD values of the migration times for RAA-I and RAA-II in different samples ranged from 1.0% to 2.5% and from 0.8% to 2.1%, respectively. The RSD values for the peak heights corresponding to RAA-I and RAA-II were about 8.0% and 10.0%, but some of them were greater than 10.0%, mainly due to matrix effects and extremely low amounts of the AAs. We note that the molar concentrations of 1.0 ppm AA-I and AA-II are 1.2×10^{-8} M and 1.3×10^{-8} M, respectively. Although the present CE-LIF approach is sensitive and efficient, we could not rule out possible positive errors due to co-migration of other RAAs; there are more than 13 AA-related compounds found in real medicinal plants [22]. However, we could not test the possibility because they are unavailable in this lab.

Based on the data listed in Table 3, we conclude: (1) the contents of AA-I in various samples are much higher than those of AA-II in most cases; (2) the species of medicinal plants has an important impact on the distribution of the contents of AAs; (3) the samples from patients generally contain higher concentrations of AAs; (4) the data may support the connection of AAs to end-stage renal failure. We also conclude that the present CE-LIF approach is sensitive, simple, efficient, and accurate for the determination of RAAs in real samples.

4. Conclusions

The RAAs reduced from OAAs in 10.0 mM HCl containing iron powder exhibit fluorescence at 477 nm when excited at 390 nm. By applying CE-LIF, the analysis of AA-I and AA-II was completed within 12 min, with the LODs at 8.2 and 5.4 nM, respectively. Having the advantage of high sensitivity and resolving power, the present CE-LIF allows detection of trace AAs in a number of medicinal samples and dietary supplements. The successful analysis of 61 medicinal samples and dietary supplements shows that the present CE-LIF is practical for the determination of AA-I and AA-II in real samples, with the advantages of simplicity, rapidity, and reproducibility. However, more cases study may be needed in order to gain more insights into the fetal effects of AAs. In order to further improve the quantity for the analysis of AAs in trace levels, stacking techniques such as sweeping and using polymer solution should be worthy trying [36,37]. It is also interested in developing new techniques that allow analysis of DNA-AA adducts and provide greater resolving power for the analysis of various AAs.

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