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Review

Liquid chromatography of active principles in *Sophora flavescens* root

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Abstract

Herbal medicines were one of the major resources for healthcare in earlier stages, and some traditional herbal medicines have been in use for more than 2000 years. Currently, they are attracting more and more attention of the modern pharmaceutical industry, as scientists has become aware that herbs have almost infinite resources for medicine development. This review provides an overview of the analytical approaches applied in the researches concentrated on various aspects of the matrine-type alkaloids in *Sophora flavescens* root. Emphasis will be laid on the analytical processes of high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), as well as gas chromatography (GC) methods. The sample extraction, separation and detection have been summarized. In addition, the applications of chromatographic determinations are introduced for the main matrine-type alkaloids in *S. flavescens* root, such as matrine, sophoridine, sophocarpine, lehmannine, sophoramine, oxymartine, oxysophocarpine, cytosine and aloperine. The advantages and limitations of HPLC, CE and GC methods in the analytical applications of the alkaloids are also discussed.

Keywords: Matrine-type alkaloids; *Sophora flavescens* root; Chinese medicines

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Abbreviations: SDS, sodium dodecyl sulfate; MT, matrine; SR, sophoranol; SRI, sophoridine; SC, sophocarpine; LMN, lehmannine; SRA, sophoramine; OMT, oxymartine; OSC, oxysophocarpine; CS, cytosine; APR, aloperine; HPLC, high-performance liquid chromatography; CE, capillary electrophoresis; HPCE, high-performance capillary electrophoresis; GC, gas chromatography; MIMs, molecularly imprinted microspheres; TLC, thin layer chromatography; LLE, liquid–liquid extraction; SPE, solid-phase extraction; MS, mass spectrometry; MISPE, molecularly imprinted solid-phase extraction sorbents; MIPs, molecularly imprinted polymers; HSCCC, high-speed counter-current chromatography; pH-ZRCCC, pH-zone refining counter-current chromatography; R.S.D., relative standard deviation; ECL, electrochemiluminescence; IPs, ionization potentials; CZE, capillary zone electrophoresis; UV, ultraviolet

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1. Introduction

Medicine of matrine series, the dried roots of *Sophora flavescens* Ait, recorded in the medicine literature *Sheng Nong's Herbal Classic* as a moderate medicine, is cold by nature with bitter taste, entering three channels of heart, spleen and kidney. The medicine is said to be able to "deal primarily with stagnation of qi in the chest and abdomen and traces of lump retention", "stabilize the five internal organs and mind, and replenish vital essence". According to *Herbal Classic of Hundreds of Medicinal Materials*,*Sophora* root is especially used to treat hotness in the heart with bitter entering heart and cold removing internal hotness. With the development of separating and extracting techniques, intensive investigations and researches have been conducted in China and it has been found that matrine-type alkaloids of the same kind are present in *S. flavescens* Ait, *Sophora alopecuroides* and *Sophora subprostrata* [1]. Matrine-type alkaloids can obviously inhibit varied clinical gastric mucosa damages. This may result from the direct neutralization of external hydrochloric acid and gastric acid by matrine, and the mitigation of matrine has certain protective effects on gastric mucosa [2]. By means of inhibiting enterocinesia, matrine-type alkaloids have visible effects on antidiarrheal [3]. They have the effects of killing amoebas and giardia lamblia stiles [4]. Matrine can kill trichomonads and slow down the development of subcutaneous abscess caused by the parasites, and also cure infections caused by mouse vaginal trichomoniasis. It is indicated that matrine can be applied as potential medicine for killing parasites, but the mechanism is not quite clear at the present [5]. Studies have shown that some matrine-type alkaloids present positive myotome effects on cardiac muscle, such as oxymatrine, sophoridine, sophocarpine and matrine which carry the common molecular structure of O=C=N–C–C–C–N (otherwise, present negative myotome effects). It has been held that this kind of structure might be the group accounting for the positive myotome effects in matrine-type alkaloids, which may be related to the activation of calcium channel [6].

Due to the high pharmacological activities of matrine-type alkaloids in *S. flavescens* root, the herb has recently drawn attention in natural medication researches. Several methods such as high-performance liquid chromatography (HPLC), high-performance capillary electrophoresis (HPCE), gas chromatography (GC) and thin layer chromatography (TLC) have been applied to the separation and determination of matrine-type alkaloids in *S. flavescens* root. Undoubtedly, HPLC is the most widely used separation technique for this application for its simplicity and general applicability to matrine-type alkaloids [7–10]. Moreover, HPCE techniques can also be used when HPLC is not suitable or efficient for the samples of interest. Sample preparation is the most important part in the application of HPLC or HPCE. Some extraction including liquid–liquid extraction (LLE), solid-phase extraction (SPE) or other methods can be selected according to the determination requirement in precision, accuracy and reproducibility. This review summarizes the analytical methods of sample preparation, HPLC separation, detection and evaluation methods for the analysis of matrine-type alkaloids in various types of samples.

2. Chemical components and contents of matrine-type alkaloids in *Sophora flavescens* root

The dried roots of *S. flavescens* contain flavones series such as kuraridin, kurarinone, isokurarinine, norkurarinone, pterocarpin, formononetin, trifolirhizin, daidzein, umbelliferone, maackiain, kuraridinol, kurarinol, neo-kurinol, and norkurarinol [11–15], but the pharmic effective compositions are mainly matrine-type alkaloids. Matrine-type alkaloids are derived from *S. flavescens* Ait, *S. alopecuroides* and *S. subprostrata*. A previous study found the alkaloid constituents of matrine-type alkaloids in *S. flavescens* root is quinolizidine and bears unique tetracyclo-quinolizindine, showing that the presence of (+)-matrine, (+)-matrine *N*-oxide, (−)-sophocarpine, (+)-sophocarpine *N*-oxide, (−)-sophoridine and (+)-sophoramine is the main alkaloids [16], which are contained equally in the seeds and the aerial and ground parts of the plant [17]. From the seeds of *S. flavescens*, which were collected in Yunnan province, China in June 1993, Ohmiya et al. isolated several new kinds of alkaloids including martine-type alkaloids, (−)-14 (β-hydroxymatrine, (−)-12 (β-hydroxysophocarpine and (−)-9α-hydroxymartine. The molecular formula and chemical structures of some main alkaloids are determined by IR, ^{1}H NMR and ^{13}C NMR as shown in Fig. 1. Moreover, the contents of the main alkaloids in the roots, aerial parts and seeds of *S. flavescens* have been compared (Table 1). (+)-Sophocarpine *N*-oxide, (+)-matrine *N*-oxide, (−)-sophocarpine, (+)-matrine and (−)-sophoridine are the main alkaloids in *S. flavescens* root, aerial parts and seeds, and possesses more than 98% of the total alkaloid. The contents of the alkaloids in seeds are especially high compared with that of root and aerial parts. *S. flavescens* roots of different areas carry different contents of alkaloids (Table 2) [18]. For instance, the produce from North Korea bears highest contents of oxymatrine, matrine

Table 1
Alkaloid contents in roots, aerial parts and seeds of *Sophora flavescens*

Alkaloids	Roots (%/fr. wt.)	Aerial (%/fr. wt.)	Seed (%/fr. wt.)
(−)-Sophocarpine	0.13	0.038	1.71
(+)-Matrine	0.32	0.080	0.71
(−)-14β-Hydroxymatrine			5.5×10^{-3}
(+)-Lupanine			18.1×10^{-3}
(−)-Sophoridine			0.38
(−)-5,6-Dehydrolupanine			8.2×10^{-3}
(−)-9α-Hydroxysophocarpine			42.3×10^{-3}
(−)-9α-Hydroxymartine			12.5×10^{-3}
(−)-12β-Hydroxysophocarpine			23.3×10^{-3}
(−)-14β-Sophoridine			29.2×10^{-3}
(+)-Matrine *N*-oxide	0.12	0.12	3.62
(+)-Sophocarpine *N*-oxide	0.073	0.097	5.05

Table 2
Matrine-type alkaloids contents of *Sophora flavescens* root (%)

Area	OMT	MT	SC	SR	TR	MA
Henan	1.935	0.252	0.733	0.378	0.095	0.031
Zhejiang	0.784	Trace	0.186	Trace	0.104	0.063
Sichuan	0.908	0.237	0.483	0.502	0.214	0.095
Guizhou A	1.415	0.091	0.339	0.369	0.091	0.044
Guizhou B	0.948	0.120	0.196	0.211	0.105	0.032
Guizhou C	1.218	0.094	0.203	0.206	0.131	0.071
Guizhou D	1.427	0.070	0.176	0.221	0.097	0.032
Guangxi	0.471	Trace	0.194	0.297	0.105	0.068
Fujian	0.794	Trace	0.078	0.096	0.044	0.014
North Korea	3.221	0.169	0.389	0.110	0.365	0.028
Unknown	2.970	0.150	0.448	0.166	0.216	0.048

and sophocarpine, while the produce from Guanxi and Fujian has the lowest content. The determination results of alkaloids in *S. flavescens* root from Gansu province and Ningxia Municipalities have been reported [19]. The contents of alkaloids in the different provinces appear also differently from 0.2237 to 0.3076 mg/g for cytosine, 0.0045 to 0.03828 mg/g for sophoramine, 0.1325 to 0.1046 mg/g for sophocarpine, 0.2844 to 0.3268 mg/g for matrine, 1.356 to 1.330 mg/g for oxymatrine, respectively. According to the determination results of Song et al. [20], the content ranges of matrine-type alkaloids found in herb samples in Hebei and Zhejiang province of China, are from sophoridine 0.36 to 2.75 mg/g, matrine 0.47 to 1.2 mg/g, sophocarpine 0.31 to 0.41 mg/g, oxymatrine 2.76 to 5.96 mg/g, oxysophocarpine 2.75 to 16.4 mg/g. Obviously, the content of oxysophocarpine is the highest and shows the largest difference among the samples. The contents of matrine in *S. flavescens* root are quite different in comparison of the number of growing years and the means of propagation according to the report of Hu et al. [21]. Obviously, the content of matrine in *S. flavescens* root increases with the lengthening of growing years, and higher content of matrine could be obtained in asexual propagation (Table 3).

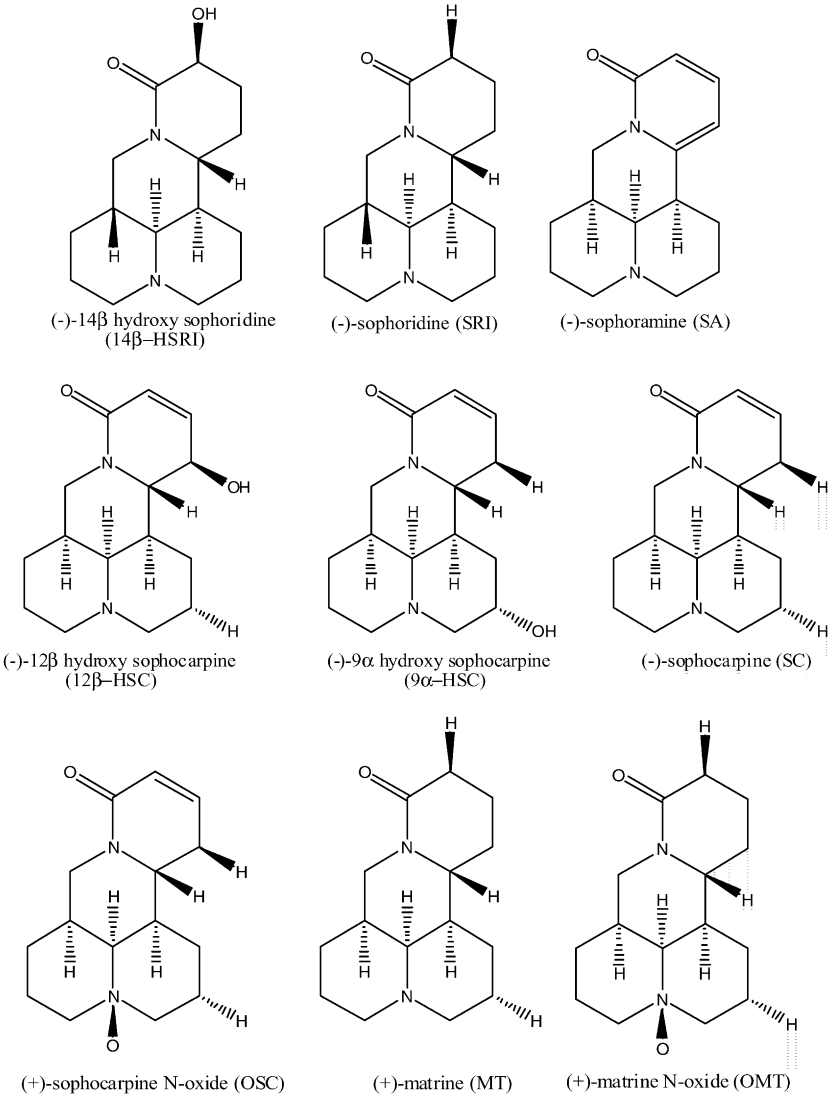

Fig. 1. Chemical structures of main matrine-type alkaloids in *Sophora flavescens* root.

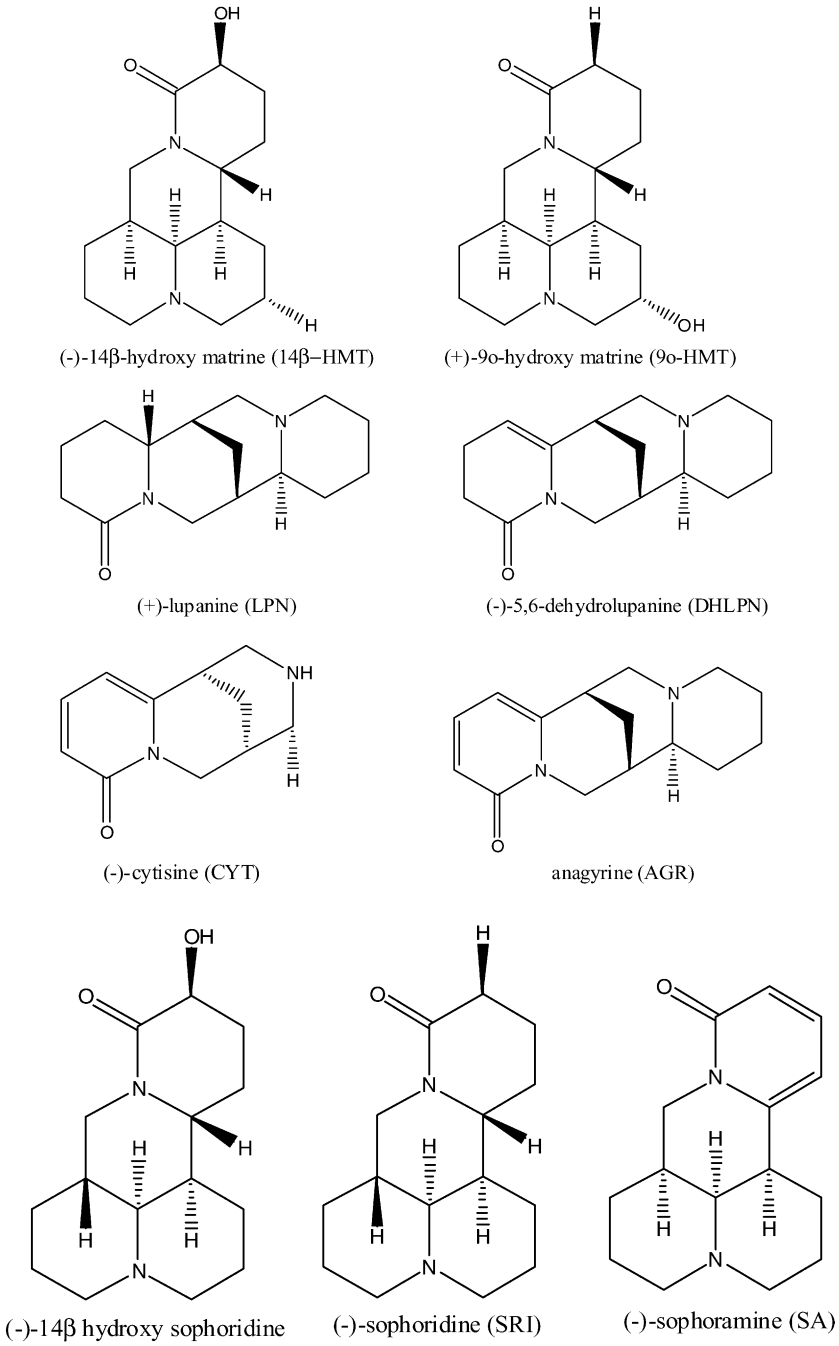

Fig. 1. (*Continued*).

Recently, some newer kind of alkaloids have been found and identified as lehmannine [22] and nicotine [23] by Liu and Zhang.

Some dissociation constants of matrine-type alkaloids as matrine, sophocarpine, sophoridine, oxymatrine and oxysophocarpine, were determined by capillary zone electrophoresis (CZE) with an untreated fused-silica capillary of 48.5 cm (40 cm to the detector, 50 μm i.d.) [24]. Under the following experimental conditions, acetate (pH 3.81–5.55) and phosphate buffer (pH 6.05–9.29) with ionic strength set

Table 3
Comparison of matrine contents in different *Sophora flavescens* root

Growth year	Matrine content (%)	
	Sexual propagation	Asexual propagation
First	1.08	1.74
Second	1.71	2.90
Third	2.30	3.00

as 0.03, alkaloids were separated within 10 min. Based on the equations of 1 and 2, the dissociation constant (pK_a) of matrine-type alkaloids is matrine 7.72, sophocarpine 7.22, sophoridine 6.51, oxymatrine 5.77 and oxysophocarpine 5.92, respectively.

3. Sample preparation

Sample preparation is probably the most important part of quantitative and qualitative analysis of matrine-type alkaloids in *S. flavescens* root. It is a critical step in chromatographic analysis, usually being the slowest step in the analysis. Recently, although high-performance liquid chromatography coupled to mass spectrometry (MS) has been developed for the measurement of effective compositions of Chinese medicines, there are few reports on the analytical application to *S. flavescens* root since the MS method requires a complex procedures, such as nebulization and vaporization of liquid, ionization of sample, removal of the excess solvent, and transfer of the ionic molecular into the mass analyzer. Moreover, the expensive instrument of MS and experiential operation limit its broad use in most research laboratories. Undoubtedly, the sensitivity HPLC with ultraviolet (UV) detection is the most popular separation technique for the analysis of matrine-type alkaloids in *S. flavescens* root. For HPLC-UV application, many sample preparation techniques have been developed for processing complex samples. Among them, liquid–liquid extraction and solid-phase extraction are the most widely employed techniques in preparing herbal samples for chromatographic analysis.

Generally, herbal samples are homogenized or blended with a water-miscible organic solvent such as methanol, acetonitrile, acetone, or chloroform. In these cases, the insoluble material removed from the mixture should then be further rinsed by the solvent several times before analysis by HPLC. Liquids used in liquid–liquid extraction (LLE) can be evaporated away, and the residue then re-dissolved in a small volume of liquid. Hence, most of LLE applications in herb samples described are off-line (Fig. 2). LLE is commonly used for sample cleanup and preparation in the chromatographic analysis for *S. flavescens* root [25–27]. In general, the selection of a solvent affects the efficiency of extraction. The amount of a solvent, extraction time and circumfluence times in the extraction process produce obvious difference in extraction rate. The efficiency of alkaloid extraction will be increased with a higher concentration of methanol applied, companying with a higher concentration of aliphatic impurity. The alkaloids are weakly basic compounds. Most of them may be solved in acid solution. If adding a base to the aqueous phase to raise the pH to about 9.5–11, the alkaloids can be extracted with chloroform. In general, the two-phase solvent system is composed of chloroform–methanol–water (4:3:2, v/v/v) in which the water has different acidities, controlled by phosphate buffer solution or hydrochloric acid [28]. Another similar preparation procedure has been introduced by

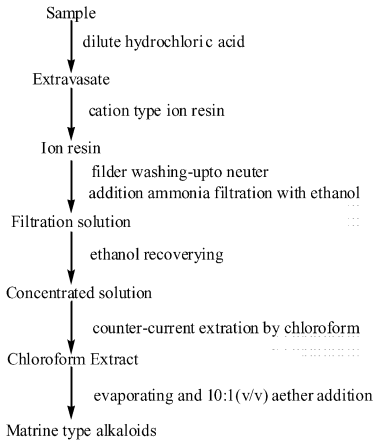

Fig. 2. Extraction process for matrine-type alkaloids from *Sophora flavescens* root.

Ito et al. [29]. Raw root of *S. flavescens* was extracted several times by a definite volume of 95% ethanol. Then, the extract were combined and evaporated to dress under reduced pressure. The residue obtained from the combined extracts is dissolved with 2% hydrochloric acid. After filtration, the acidic aqueous solution is extracted by chloroform. Before the total alkaloids are extracted by chloroform, the pH of the aqueous should be adjusted to 9.6 with sodium hydroxide. In the last step, the chloroform extracts are combined and evaporated to dryness.

Solid-phase extraction is a commonly employed sample preparation method in complex matrices. Differences in affinity of various molecules to active sites located on the surface of sorbent materials resulting in the separation of mixtures of different molecules are the basic principle of SPE. SPE is used to selectively remove interfering matrix components, leading to the reduction of chromatographic analytical time and the improvement of assay selectivity, accuracy and sensitivity. Recently, molecularly imprinted solid-phase extraction sorbents (MISPE) were extensively used to clean up the impurities in environmental and biological samples [30,31] and to enrich effective components from complicated media [32], since MISPE compared to enzymes possess several advantages including physical robustness, high strength, and resistance to elevated temperature, pressure, erosion by acid, base, metal ions and organic solvents [33–35]. Molecularly imprinted polymers (MIPs) were employed as SPE sorbent for chromatographic separation and the determination of matrine and oxymartine from *S. flavescens* by Lai et al. [36]. In this application, MIPs are cross-linked polymers with specific binding size. MIPs for matrine and oxymartine recognition are tailor-made in situ by the co-polymerization of the crosslinker of poly(vinyl alcohol) and functional monomer of methacylic acid in the presence of template molecules of matrine and oxymartine. After removal of the template molecules from the polymers, the recognition sites are complementary to the template molecule of matrine and oxymartine. The molecularly imprinted microspheres (MIMs)

using matrine as template molecule are proposed and synthesized by aqueous micro-suspension polymerization. The MIMs obtained are employed as SPE sorbent to separate matrine from *S. flavescens* and pack it into a stainless steel column as chromatographic stationary phase to determine matrine.

As the yield of tradition extraction methods is less satisfactory, a new extraction method has recently been developed using microwave energy for the extraction of matrine-type alkaloid from ground lupin seeds [37]. In the microwave extraction, a 0.5 g of ground seeds was suspended in a screw-capped vial with 3 ml solvent containing methanol–acetic acid (99:1, v/v). To avoid boiling of the solvent, the irradiation was interrupted after 30 s. The sample was cooled to room temperature in 2–8 min and then irradiated and cooled again if necessary. In comparison with the traditional shaken flask extraction, by which a 5 g amount of ground lupin seeds was suspended in 10 ml of methanol–acetic acid (99:1, v/v) and shaken in a 100 ml erlenmeyer flask for 20 min, the microwave extraction provided 20% higher efficiency than that of shaken flask extraction, and achieved considerable decreases in time and solvent consumption. Generally, the yield of the traditional extraction method using the above solvent was about 52%, but the microwave extraction with the same solvent system yielded about 80%.

4. Separation and quantitation

HPLC is primarily an analytical separation technique, used to detect and quantitate analytes of interest in more or less complex mixtures and matrices. Its ability to separate thermally labile and non-volatile analytes has made HPLC the method of choice for the analysis of most drug compounds, including the active compositions of herbs. In reversed-phased chromatography, a non-polar stationary phase is used in conjunction with polar, mainly aqueous mobile phase. Seventy and 80% of all HPLC applications utilize this technique. Its popularity is based largely on its ease of use; equilibration is fast, retention times are reproducible, and the basic principles of the retention mechanism can be easily understood. Most stationary phases are silica-based bonded phase, but polymeric phase, phases based on inorganic substrates other than silica, and graphitized carbon have found their place as well. Many matrine-type alkaloids from *S. flavescens* root are relative polar, which has made reversed-phase HPLC a suitable method for their determination. The most popular reversed-phase columns for lupin alkaloids separation today are applied from silica-based bonded phase, and among those the C_{18}-type bonded or amino-bonded phase is most frequently used (Table 4 [8,9,38–52]). Matrine-type alkaloids from *S. flavescens* root were first isolated and reversed-phase HPLC separation by Ota et al. [53]. In this work, matrine, oxymatrine, sophoranol, sophoranol *N*-oxide and anagyrine were isolated from Chinese *S. flavescens* root. Sophoranol *N*-oxide and anagyrine were quantificationally detected on an HPLC column Lichrosorb NH_2 with a MeCN-H_3PO_4 aqueous solution (pH 2.0) mobile phase system, and matrine, oxymatrine, sophoranol were separated by using pH 1.3 MeCN–H_3PO_4 aqueous solutions–EtOH system. It was found that the Chinese and Korean *S. flavescens* roots apparently differed from the dry root of Japanese *S. flavescens* root in the contents of these alkaloids. Saito et al. [54] reported an HPLC method to devise the qualitative and quantitative analysis of lupin alkaloids in plants. Three solvent systems consisting of diethyl ether, methanol and ammonia solution and reversed-phased chromatography with a buffered aqueous solution containing acetonitrile were selected for the separation of 22 naturally occurring matrine-type alkaloids. The elution of alkaloids was monitored by UV absorption at 220 and 310 nm. In Jin's HPLC system [10], a Lichrosorb-NH_2 column (4.0 mm × 250 mm) and CH_3CN–H_3PO_4 (pH 2)–CH_3CH_2OH (80:8:10) as mobile phase were applied for five kinds of matrine-type alkaloids from *S. flavescens* root, matrine, oxymatrine, sophoridine, sophocarpine and oxysophocarpine separation. The method was efficient and took only 15 min for one run. According to the report of Li et al. [41], separation of six lupin alkaloids matrine, oxymatrine, sophoridine, sophocarpine, sophoramine and aloperine of *S. flavescens* root obtained from Anhui province, China by a Nova park C_{18} column (3.9 mm × 150 mm) was achieved in an optimal mobile phase containing 70% of methanol and 30% of 10 M KH_2PO_4 buffer (pH 5.1) and 0.25 ml/l triethylamine. Recently, a simple HPLC methodology for the simultaneous separation of three alkaloids, matrine, sophoridine and oxymatrine, in the sample of Chinese *S. flavescens* root has been developed [38] in which the processed chromatographic conditions included a Kromasil C_{18} silica column (250 mm × 4.6 mm i.d.; particle size 5 μm). The contents of the mobile phase for that separation were 0.01 M KH_2PO_4 buffer–methanol–triethylamine (94:6:0.01, v/v/v). The temperature of the column was maintained at 40 °C. The three alkaloids could be simultaneously separated with minimal interference from the endogenous components in the sample. Matrine, sophoridine and oxymatrine had retention times of approximately 9.4, 12.2 and 20.8 min, respectively. Typical chromatograms of standards and sample containing the alkaloids are shown in Fig. 3. In the study of Li et al. [41], the composition and concentration of organic component in the mobile phase significantly affected the chromatographic separation of the matrine-type alkaloids. With increasing the buffer or decreasing methanol content, the retention times of the alkaloids were obviously prolonged, but the responses of peak area changed only slightly with varying composition of the mobile phase. The changed tendency was the same if methanol in the mobile phase was replaced with acetonitrile, but the effect was more conspicuous. Many, if not most, of pharmacologically active compounds including matrine-type alkaloids are amines. The principal analytical issue with amine has often to do with mixed-mode interaction between the stationary support and the analytes. Incompletely endcapped silica-based phases may exhibit tailing. The use of ion pairing or ion suppression is common in

Table 4
HPLC applications on the separation and determination of matrine-type alkaloids of *Sophora flavescens* root

Objective	Alkaloid	Mobile-phase (v/v)	Separation column	Linearity	Recovery (%)	Reference
Sophora flavescens Ait	MT, SR, OMT	0.01 M KH_2PO_4 buffer–methanol–triethylamine (94:6:0.01)	Kromasil C_{18}	MT: 0.2–120.0 μg/ml	MT: 93.9	[38]
				SR: 0.2–115.2 μg/ml	SR: 95.3	
				OMT: 0.2–110.4 μg/ml	OMT: 93.5	
Human plasma	MT, OMT, SR	Acetonitrile–absolute ethyl alcohol–phosphoric acid (80:10:8)	Lichrosorb NH_2		MT: 106.96	[39]
					OMT: 105.04	
Cream	MT	Methanol–3.5% SDS (7:2)	Phenomenex μl Tracar B 5 DOS(20)	20–120 μg/ml	96.0	[40]
	MT, OMT, SR, AR, SC, SRI	10 mM KH_2PO_4–methanol (3:7)	Nova Pak C_{18}			[41]
Sophora flavescens Ait	MT, SR	Methanol–H_2O–triethylamine (55:45:0.02)	Hypersil C_{18}	1.0–10 μg/ml	97.1	[42]
Sophora tonkinensis gapnep	MT, OMT	Acetonitrile–absolute ethyl alcohol–phosphoric acid (80:10:10)	YWG–NH_2	MT: 0.2–2.5 μg/ml	MT: 100.2	[43]
				OMT: 0.4–4.5 μg/ml	OMT: 98.6	
Matrine phosphate injection	MT	Acetonitrile–0.1% phosphoric acid (10:90)	ZORBAX SB-C_{18}	12.65–101.20 μg/ml	100.30	[44]
K562 culture-medium	MT, OMT	0.05 M NaH_2PO_4–acetonitrile–methanol		20–180 μg/ml		[45]
Jieeryin, Fuyanjie	MT, OMT	Methanol–H_2O–triethylamine (55:45:0.02)	Shim-pack CLC-ODS	OMT: 0.05–3.5 g/ml	MT: 96.9	[8]
				MT: 0.025–2.5 g/ml	OMT: 98.9	
Fufang guangdougen heji	MT	Methanol–H_2O (95:5)	Si	4 ∼ 100 μg/ml	97.74	[46]
Pure samples	SR, SC, SRI, MT	80 mM NaH_2PO_4–K_2HPO_4 buffer–acetonitrile (7:3)	ODS-80 Ts reversed-phase column	SRI: 2×10^{-10} to 5×10^{-5} g/ml MT: 5×10^{-10} to 5×10^{-5} g/ml SR: 8×10^{-10} to 7×10^{-5} g/ml SC: 2×10^{-9} to 6×10^{-5} g/ml		[47]
Kushensu	OMT, OSC	0.05 M NaH_2PO_4–acetonitrile–methanol–sodium perchlorate (910:50:10:20)	Alltech C_{18}	0.02 ∼ 0.80 μg/ml		[48]
Kushensu injection	OMT, OSC	Acetonitrile–absolute ethyl alcohol–phosphoric acid (80:10:8)	Spherisorb NH_2	OMT: 0.04–0.12 mg/ml	OMT: 98.8	[49]
Antivirus granule	MT	Acetonitrile–0.025 M $(NH_4)_2SO_4$–SDS 35:65:0.6 (g)	ODS reversed-phase column	0.06–2.44 μg	97.73	[50]
Compound radix euphorbiae fischerianae injection	MT	Acetonitrile–0.02 M NaH_2PO_4 (60:40)	Shim-pack CLC-ODS	0.15–0.90 mg/ml	99.13	[51]
Compound oral liquor	MT	Methanol–H_2O (95:5)	YWG–SiO_2	4.0–100 μg/ml		[9]
Antidiarrhea tablet	MT, SC, SR	Acetonitrile–absolute ethyl alcohol–phosphoric acid (80:10:8)	Spherisorb NH_2	SR: 0.05–0.15 mg/ml	101.2	[52]

the analysis of matrine-type alkaloids. A method for determination of matrine in cream by micelle HPLC was established by Wei et al. [40]. A Phenomenex μL Tracar B 5 DOS (150 mm × 4.6 mm i.d.; particle size 5 μm) column was used with the mobile phase of methanol–H_2O (7:2, v/v)–3.5% dodecyl sulfate sodium (SDS). The addition of SDS in the mobile phase increased the separation efficiency, and decreased the peak tailing. Furthermore, the addition of a little triethylamine as a pH regulative reagent in the mobile phase could obviously increase the peak symmetry and sharpened the peak, which improved the separation and sensitivity of the alkaloids [41,42], the retention times of the alkaloids tended to decrease with the increase of the concentration of triethylamine. Generally, the separation and peak worsened when triethylamine content was increased to over 0.03% [63] or 2.5 ml/l [41].

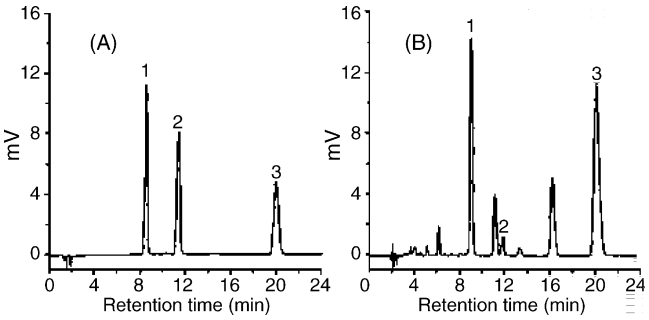

Fig. 3. Chromatograms of: (A) mixture of matrine, sophoridine and oxymatrine; and (B) *Sophora flavescens* Ait. Samples collected in Anhui province, China. (1) Matrine (9.4′); (2) sophoridine (12.2′); (3) oxymatrine (20.8′). Reprinted with permission from ref. [38].

Recently, high-speed counter-current chromatography (HSCCC) has become an attractive method for the preparative separation, and been applied in the measurement of most alkaloids from some crude extracts of Chinese traditional medicinal herbs [28], since HSCCC provides many important advantages over the conventional counter-current chromatographic method including an over 10-fold increase in sample loading capacity, high concentration fraction, concentration of minor impurities, etc. HSCCC is a liquid–liquid partitioning chromatography method in which the stationary phase is immobilized by a centrifugal force. Sample components are partitioned between the mobile and stationary phases and separated on the basic of differences in their partition coefficients when the mobile phase is pumped through. In general, matrine-type alkaloids are all weakly basic compounds. A pH-zone refining counter-current chromatography (pH-ZRCCC) was advanced for the separation of matrine-type alkaloids of *S. flavescens* root by Yang et al. [29] based on the pH character of lupin alkaloids. In pH-ZRCCC, a retainer base (or acid) in the stationary phase was applied to retain the analytes in the column, and an elute acid (or base) was used to elute the analytes according to their pK_a values and hydrophobicity. For pH-ZRCCC separation, the column made of a 110 m × 1.6 mm i.d. PTFE tube with a total capacity of 240 ml was first entirely filled with the organic stationary phase. The sample solution was injected through a sample injector valve with a 20 or 30 ml loop. The aqueous mobile phase was pumped through the column at a flow-rate of 1.5 ml/min while the column was rotated at 800 rpm. A Model 8823A-UV detector at 254 nm and an Orion Model 333 pH monitor were continuously used to monitor the effluent from the outlet. A typical pH-ZRCCC chromatogram obtained for the separation of crude alkaloid of *S. flavescens* root is presented in Fig. 4.

HPLC is the most widely employed technique in the analysis of lupine alkaloid of *S. flavescens* root. In their UV detection, a wavelength about 202–220 nm [38,39,55,56] is generally selected for high sensitivity. In Li's report [41], a HPLC-UV method was described for the simultaneous determination of matrine, sophoridine and oxymatrine in *S.*

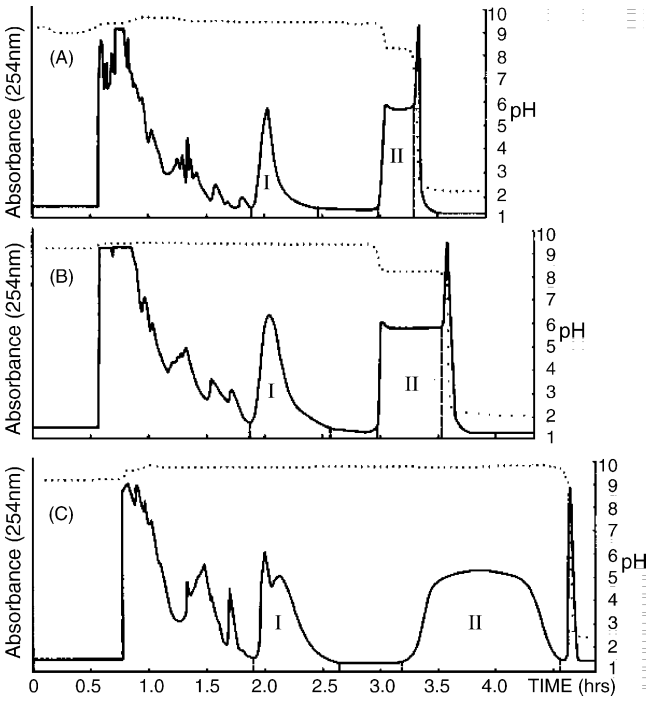

Fig. 4. Chromatograms of the crude alkaloid extract from the root of *Sophora flavescens* Ait obtained by pH-zone-refining CCC. Experimental conditions were as follows: solvent system: methyl *tert*-butyl ether–water (1:1); stationary phase: upper phase (10 mM triethylamine); mobile phase: lower phase (10 mM HCl for A and B, 5 mM for C); flow-rate: 1.5 ml/min; sample size: 1.0 g (A), 2.0 g (B) and 1.0 g (C) dissolved in 20 ml (A), 30 ml (B) and 20 ml (C) of each phase, respectively; revolution speed: 800 rpm; retention of stationary phase: 66%. I: matrine, II: sophocarpine. Reprinted with permission from ref. [29].

flavescens root from different areas of China, such as Shanghai, Anhui and Jiangsu. The results showed that the percentage of relative standard deviation (R.S.D.) of the mixture assays and average recoveries of the three alkaloids were 0.24–0.49% and 93.9% for matrine, 1.08–1.89% and 95.3% for sophoridine, 0.23–0.65% and 93.5% for oxymatrine. A method of determination matrine and oxymatrine in human plasma was performed by Fei et al. [39]. The alkaloids in the plasma were extracted with chloroform–*n*-butyl alcohol (98:2, v/v) after basification and purification with neutral alumina solid-phase extraction. The HPLC separation was performed on a Lichrosorb NH_2 column using CH_3CN–CH_3CH_2OH–H_3PO_4 (80:10:8, v/v/v, pH 2.0). Due to the high pharmacological activities of matrine-type alkaloids of *S. flavescens* root, the herb has recently become one of the focus of attention in natural medication researches. A numbers of HPLC systems have been reported for the determination of matrine-type alkaloids in Chinese medicines or correlative products. Typical chromatograms of matrine and oxymatrine in Chinese gynaecologic medicines are named Jieeryin, Fushuanyan and their standards are shown in Fig. 5 [8]. A 10 ml Jieeryin sample was adjusted to pH 9–10 by 14% ammonia solution, and then extracted four times with 20 ml chloroform. An exact volume of 10 ml extraction solution was purified by a small column packed neutral alumina

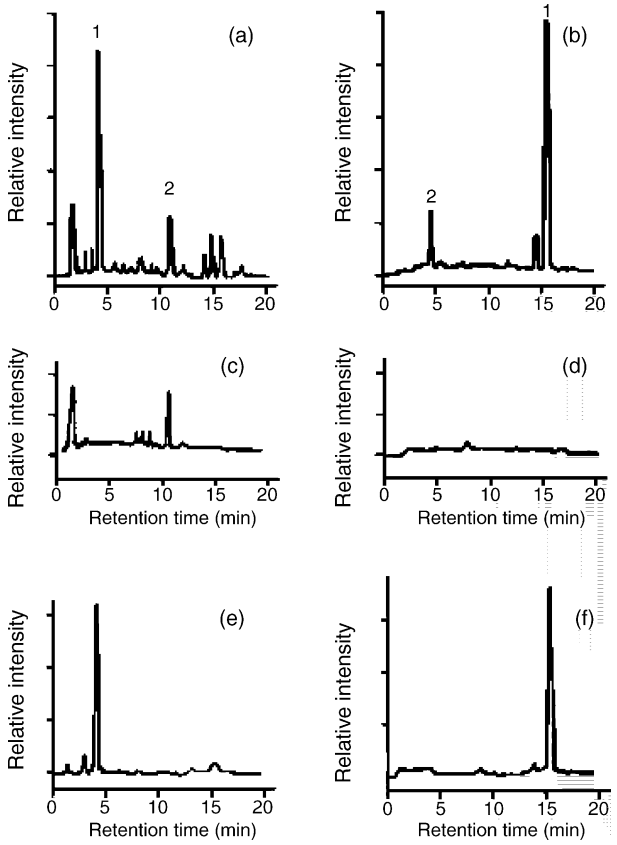

Fig. 5. Typical chromatograms of Chinese gynaecologic medicines and their standards: (a) Jieeryin sample; (b) Fuyanshuan sample; (c and d) corresponding blanks; (e) standard oxymatrine solution; and (f) standard matrine solution. (1) Oxymatrine; (2) matrine. Reprinted with permission from ref. [8].

(10 mm × 90 mm) with a 10 ml chloroform, and then 10 ml chloroform–methanol (7:3, v/v) solution and then gave alkaloids. The alkaloids were collected and evaporated to dryness. The residue obtained was reconstituted with mobile phase and filtered through a 0.45 μm membrane. The clear filtrate was used in as the HPLC assay. Moreover, a reversed-phased HPLC method for the determination of matrine and oxymatrine in *S. alopecuroides* L. and its preparation were reported by Wang and Yang [57]. A Shim-pack CLC-ODS column (150 mm × 6.0 mm, particle size 5 μm) and SPD-M6A PDA detector were used in this system. A typical chromatogram of alkaloids separated from *S. alopecuroides* L. is shown in Fig. 6. Compared with the compound medicines, the process and HPLC chromatograms of injections are obviously simple since less composition exists in injections (Fig. 7). Separation and determination of matrine in matrine phosphate injection was achieved with a ZORBAX SB-C_{18} column in an optimal mobile phase containing acetonitrile and phosphoric acid (10:90, v/v). The injection sample was directly injected into the HPLC system after mobile phase dilution [44].

Besides of UV detection, in recent years, the electrochemiluminescent (ECL) method using $Ru(bpy)_3^{2+}$ has become an attractive HPLC detection means for matrine and

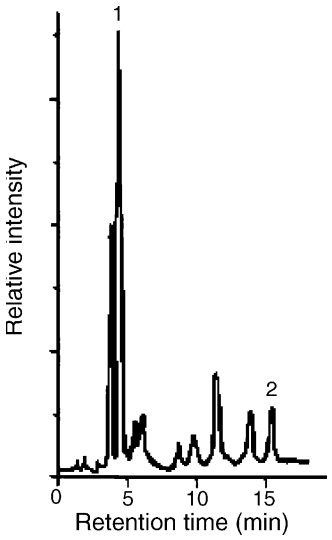

Fig. 6. Chromatogram of alkaloids separated from *Sophora alopecuroides* L.: (1) oxymatrine; (2) matrine. Reprinted with permission from ref. [57].

related matrine-type alkaloids, such as sophoridine, sophoranol and sophocarpine [47], because quinolizidine alkaloids in *S. flavescens* contain tertiary amine functional groups and therefore they could be determined sensitively while using the chemiluminescence reaction with $Ru(bpy)_3^{2+}$. Generally, the amino group present in amine or amino acid produces a secondary amine followed by a dealkylation process in the presence of water, and the intermediates of neutral amine radical have sufficient energy to react with $Ru(bpy)_3^{2+}$ or $Ru(bpy)_3^{3+}$ to yield excited state $Ru(bpy)_3^{2+}$ and hence light emission. The study identifying ECL emission of lupin alkaloid was affected by its three-dimensional conformation of hydrogen, β-carbon atom substitution group character or a carbon–carbon double bond substituent in alkaloid. The cal-

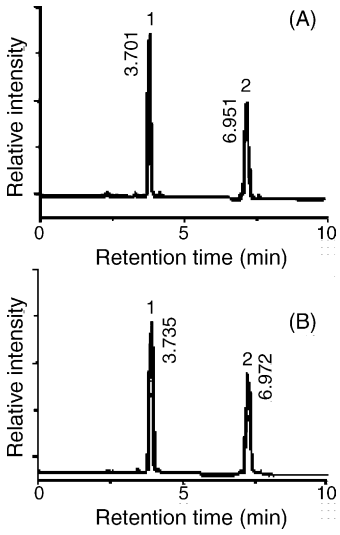

Fig. 7. Chromatograms of: (A) standard matrine solution; and (B) matrine phosphate injection. (1) Matrine; (2) internal standard. Reprinted with permission from ref. [44].

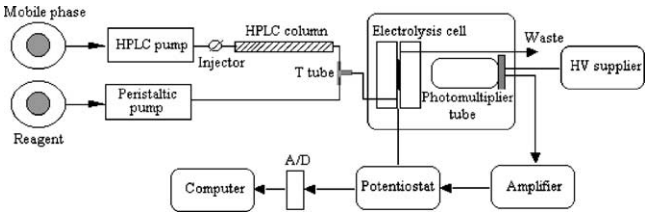

Fig. 8. Experimental setup for ECL-HPLC.

culated values of ionization potentials (IPs) in N atom and luminescence intensities for these alkaloids provided a general trend of ECL intensity for the alkylamines. Investigation results showed that the luminescence was obtained only in alkaline solution beyond pH 9.0. The supporting electrolyte also affected the electrolytic efficiency of matrine-type alkaloid, which led to different luminescence intensity. The system for matrine-type alkaloids HPLC-ECL determination is shown in Fig. 8. The main body of the thin layer electrolysis flow cell was constructed from two pieces of Diflon blocks separated by a 50 μm thick Teflon spacer. The volume of the thin layer cell was about 1.5 μl. A three-electrode system was used for potentiostatic control of the electrolytic system. The working electrode was a glassy carbon disk (22.1 mm^2). The counter electrode set at the outlet consisted of a stainless-steel pipe, and the reference electrode was Ag/AgCl (saturated KCl solution). A potentiostat was used for electrolysis. The light emission was detected using a photomultiplier tube and the signal was amplified and recorded by a chromatographic processor. The samples were separated on an ODS-80 Ts reversed-phase column (150 mm × 4.6 mm, Tosoh, Japan) with a mobile phase containing 80 mM NaH_2PO_4–K_2HPO_4 buffer solution (pH 6.0)–acetonitrile (7:3) and 40 mM sodium dodecyl sulfate. The reagent solution with 0.8 mM $Ru(bpy)_3^{2+}$ in 0.05 M NaOH–NaAc–0.3 M KNO_3 buffer solution (pH 10.0) was mixed with the elute from HPLC column before flowed into ECL detector. The detection limits ranged from 3×10^{-11} g/ml for sophoridine, 6×10^{-11} g/ml for matrine, 7×10^{-11} g/ml for sophoranol, to 1×10^{-10} g/ml for sophocarpine, respectively, at an S/N of 3. Relative standard deviations were less than 3% for 10 replicate injections for all samples. The calibration curve for the four alkaloids was found to be from 2×10^{-10} to 5×10^{-5} g/ml for sophoridine, 5×10^{-10} to 5×10^{-5} g/ml for matrine, 8×10^{-10} to 7×10^{-5} g/ml for sophoranol, and 2×10^{-9} to 6×10^{-5} g/ml for sophocarpine, respectively.

5. High-performance capillary electrophoresis and other methods

High-performance capillary electrophoresis is a separation technique of high efficiency with low sample and solvent consumption. The technique allows the separation of almost any type of compound, regardless of the chemical nature, size, conformation, or charge. The most attractive advantage of HPCE is to break the micro separation limit and make routine separation at the nano or even atto level possible, although the lower sensitivity and reproducibility compared to HPLC are crucial points for analysis of substances occurring in very low concentration. It has been widely used for separation and determination of pharmacologically interesting compounds in biological matrices such as plant. In the field of natural product analysis, HPCE has gained acceptance as an analytical technique. It is particularly useful for solving separation problems in cases that those problems are difficult or too time-consuming to be solved by HPLC. In general separation of matrine-type alkaloids from *S. flavescens*, a selection of weak acidic running buffer about pH 3.0 could be considered since the alkaloids are weakly basic compounds. An uncoated fused-silica capillary with about effective length of 50 cm is commonly used. Addition of the organic solvent resulted in the reduction of the sphere of hydration of the analytes. As a consequence, the difference in charge to size ratio increased, resulting in a better resolution of the analytes. A better separation of matrine-type alkaloids could be obtained if the running buffer contains a suitable amount of organic reagent such as THF or propanol [20,58]. In 1999, Song reported the determination of quinolizidine alkaloids in traditional Chinese herbal drugs by non-aqueous capillary electrophoresis [20]. A total of 10 alkaloids including matrine, oxymatrine, sophocarpine, oxysophocarpine, sophoridine, cytisine, sophoramine, aloperine, lehmannine and dauricine could be separated within 18 min (Fig. 9). Separations of alkaloids from *S. flavescens* (Kushen), *S. alopecuroides* (Kudouzi) and *Sophora tonkinensis* (Kugancao) were achieved in an optimal running buffer composed of 50 mM ammonium acetate, 10% tetrahydrofuran and 0.5% acetic acid in methanol using fused-silica capillary of 47.6 cm (40 cm effective length) × 50 μm i.d. The limits of detection for all 10 alkaloids were over the range of 0.93–2.31 μg/ml. The method was successful applied to the phytochemical analysis of alkaloid extracts from three commonly used traditional Chinese herbal drugs. The contents of pharmacologically active alkaloids, matrine, sophocarpine, sophoridine, oxymatrine and oxysophocarpine in different parts of *Sophora* species were quantitatively determined within 16 min [20]. Hu et al. [19] used capillary zone electrophoresis in the extract of *S. flavescens* root for the separation and quantitative determination of five alkaloids, cytosine, sophoramine, sophocarpine, matrine and oxymatrine. As shown in Fig. 10, the all alkaloids can be monitored at UV 214 nm using an uncoated fused-silica capillary of 75 μm i.d. with a total length of 57 cm (50 cm effective length). Separation was performed by a buffer of 110 mM sodium dihydrogenphosphate and 15% 2-propanol (pH 3.0) and kept in a constant voltage of 27 kV. Comparing the CZE and HPLC methods for alkaloids separation, CZE shows several advantages in higher separation performance over HPLC, such as rapidity of analysis, small sample volume, no requirement for organic solvent in the running buffer and low cost of reagents. In the above CZE method, the five alkaloids could be baseline separated in 15 min and the detection limits for the five

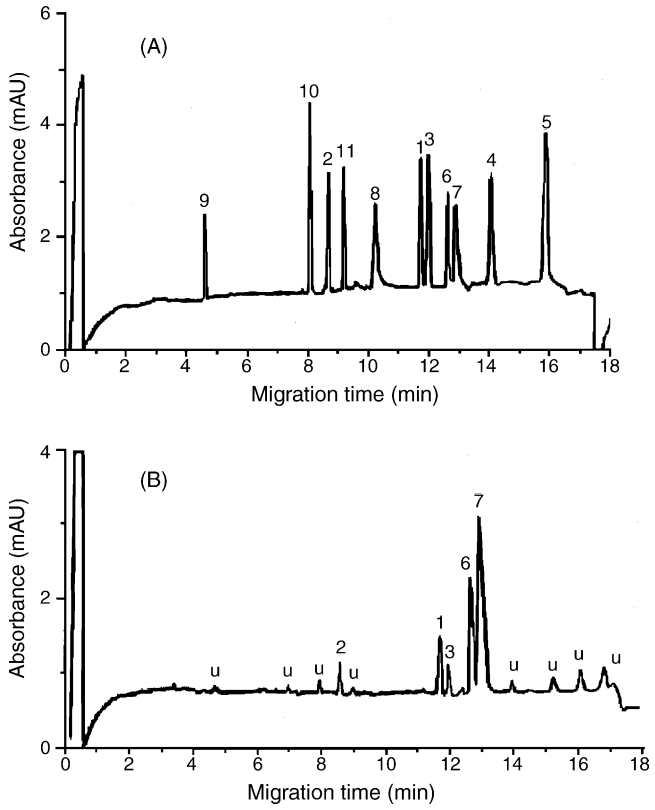

Fig. 9. Electropherograms of: (A) 10 alkaloids and the I.S. mixture; (B) *S. flavescens* applied voltage: 30 kV, temperature: 25 °C column: 47.5 cm (40 cm effective length) × 50 μm of uncoated fused-silica capillary buffer solution: 50 mM ammonium acetate, 10% THF and 0.5% acetic acid in methanol. (1) Matrine; (2) sophoridine; (3) sophocarpine; (4) lehmannine; (5) sophoramine; (6) oxymatrine; (7) oxysophcarpine; (8) cysine; (9) aloperine; (10) dauricine; (11) scopolamine (internal standard). Reprinted with permission from ref. [20].

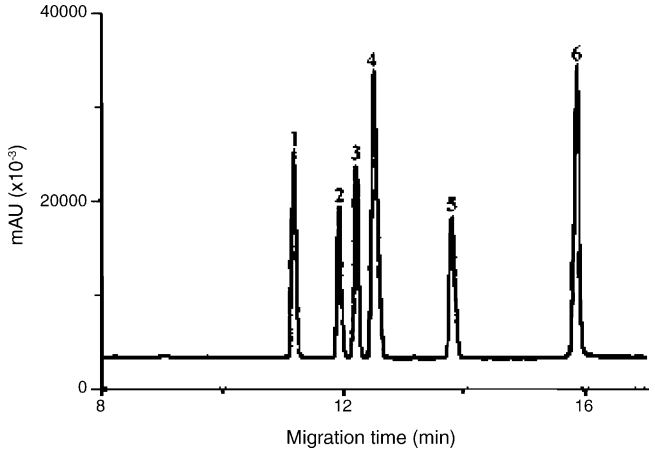

Fig. 10. Electropherogram for the separation of the alkaloids. Separation buffer, 110 mM sodium dihydrogenphosphate, 15% 2-propanol, pH 3.0. Electrokinetic injection with 8 kV and 11 s (+ to −) after preliminary pressure injection of water for 3 s. (1) Cytosine; (2) sophoramine; (3) sophocarpine; (4) matrine; (5) oxymatrine; and (6) brucine (internal standard). Reprinted with permission from ref. [19].

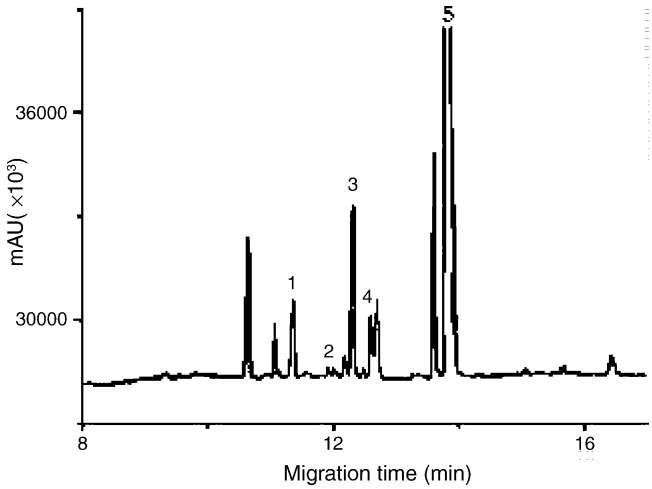

Fig. 11. Electropherogram for the sample from *Sophora flavescens* Ait. Separation buffer, electrokinetic injection parameters, compound identities same as in Fig. 10. Electropherograms of: (A) 10 alkaloids and the I.S. mixture; (B) *S. flavescens*. Reprinted with permission from ref. [19].

alkaloids on the basis of a signal-to-noise ratio of 3 were determined from a range of 0.7 to 0.9 ng/ml. For a given sample (Fig. 11), R.S.D. values of the migration times and relative peak areas were below 0.5 and 1.5%. The described HPCE methods for the separations and determinations of matrine-type alkaloids of *S. flavescens* root are summarized in Table 5 [19,20,24,58–61].

A wide range melting point of matrine-type alkaloids makes it difficult to apply capillary gas chromatography for their separation, and a few reports on this field have been found. The melted point of matrine ranges from 76 to 87 °C, but the melted point of γ-type matrine changes to 223 °C [62]. Oxymatrine monohydrate is melted at 162–163 °C, but anhydrated oxymatrine at 207 °C. Generally, the separation of matrine and other matrine-type alkaloids of *S. flavescens* root or Chinese medicines using GC have often required a temperature program. In the Jiang's report [62], a stainless steel separation column (1.0 m × 6 mm, i.d.) packed with OV-17 as stationary phase and FID detector were employed. The temperature program was applied with 150 °C for 1 min, increasing 20 °C/min to 250 °C for 3 min, then increasing 20 °C/min to 300 °C for 2 min. Recently, the separation and determination of matrine in vegetal pesticide by GC have been attracted interests since matrine mixed with nicotine has been considered as main compositions of high effective pesticide in green agriculture [63,64]. A 25 m × 0.32 mm i.d. flexible fused-silica packed with 5% Ph Me silicone capillary column or a filled column (2.5 m × 3 mm, i.d.) packed with 10% PEG-20M and FID detector was applied. The recovery and R.S.D. are ranged from 97.5 to 99.4, and 1.73 to 6.01%, respectively. A summary of GC application on the separation and determination of matrine alkaloids of *S. flavescens* root is presented in Table 6 [62,63,65,66].

Although thin layer chromatography lacks quantitative precision, sensitivity and separation efficiency compared

Table 5
HPCE applications on the separation and determination of matrine-type alkaloids of *Sophora flavescens* root

Objective	Compound	CE mode	Running buffer	Separation column	Linearity range (μg/ml)	LOD (μg/ml)	Reference
Herbal medicine	MT, SC, SRI, OMT, OSC	CZE	Acetate (pH 3.81–5.55) and phosphate buffer (pH 6.05–9.29)	Untreated fused-silica capillary			[24]
Sophora flavescens Ait	SR, SC, MT, OMT	CZE	110 mM NaH_2PO_4–15% 2-propanol (pH 3.0)	Uncoated fused-silica capillary	CS: 0.01−0.1	CS: 0.7×10^{-3}	[19]
					SR:0.01−0.1 SC: 0.01−0.1 MT: 0.01−1 OMT: 0.01−1	SR: 0.8×10^{-3} SC: 0.7×10^{-3} MT: 0.9×10^{-3} OMT: 0.8×10^{-3}	
Sophora flavescens Ait	SRI, SC, MTO, MT, OSC	CZE	0.04 M citrate buffer (pH 4.5)–50% methanol–triethylamine	Uncoated fused-silica capillary		OSC: 0.07	[58]
						SRI: 0.042 SC: 0.039 M: 0.032 OM: 0.068	
Sophora subprostata	MT, OMT	HPCE	130 mM phosphate buffer (NaH_2PO_4–H_3PO_4 pH 3.5)–acetonitrile (3:1)	Uncoated fused-silica capillary	MT: 22.0–440.0	MT: 1.1	[59]
					OMT: 10.8–216.0	OMT: 1.0	
Sophora flavescens Ait, *S. alopecuroides* L., *S. tonkinensis*, gapnep	MT, SC, OMT, SR, OSC	Non-aqueous CE	50 mM ammonium acetate–10% tetrahydrofuran–0.5% acetic acid–methanol	Uncoated fused-silica capillary	SRI: 2.51–50.1	SRI: 1.18	[20]
					SC: 2.52–50.4 MT: 2.71–54.2 OMT: 3.30–65.9 OSC: 3.10–62.0	SC: 1.05 MT: 1.27 OMT: 2.24 OSC: 2.11	
Sophora flavescens injection	MT, OMT, SC, OSC	CZE	200 mM Tris salt–40 mM NaH_2PO_4–20% isopropanol (pH 5.5)	Uncoated fused-silica capillary	SC: 0.2–5.7	SC:0.1	[60]
					MT: 0.3–16.7 OMT: 4.8–240 OSC: 1.2–59	MT: 0.14 OMT: 0.3 OSC: 0.18	
	MT, OMT	HPCE	20 mM phosphate buffer (NaH_2PO_4–H_3PO_4 pH 8.0)	Uncoated fused-silica capillary	MT: 126–1260	MT: 1.0	[61]
					OMT: 100–1000	OMT: 0.5	
Sophora flavescens Ait	MT, OMT	HPCE		Uncoated fused-silica capillary	MT: 70–350		[27]
					OMT: 50–250		

to the column methodologies, TLC methods has been developed and employed for the separation and determination of alkaloids, especially for matrine since TLC is a quick, convenient, and inexpensive technique with the ability to assay many samples in parallel on a single TLC plate, leading to a large increase in sample throughput compared to the column methods. The contents of matrine in Chinese medicines were detected using silica gel plates with chloroform–methanol–ammonia (5:0.6:0.2, v/v/v) or toluene–acetone–ammonia (10:1.5:0.5, v/v/v) as developing solvent system [67,68]. The plates were layer scanning with a UV spectrophotometer or a lamina scanner. Similar TLC methods have been also developed to the detection of alkaloids of *S. flavescens* root in Chinese medicines and relative products, and are summarized in Table 7 [67–76].

Table 6
GC applications on the separation and determination of lupin alkaloids of *Sophora flavescens* root

Objective	Compound	Separation column	Detector	Reference
	OMT	5% Ph Me silicone capillary	FID	[63]
Sophora flavescens Ait	MT, OMT	BD-1701 capillary	FID	[62]
Anti-rheumatism capsule	MT	Stainless steel	FID	[65]
Lupinus angustifolius	CS	Fused-silica OV-101 capillary	MS	[66]

Table 7
TLC application on the separation and determination of matrine-type alkaloids of *Sophora flavescens* root

Objective	Compound	Developing solvent	TLC plates	Linearity range	Reference
Ginseng angelica pill	MT	Chloroform–methanol–ammonia (5:0.6:0.2)	Silica gel G		[68]
Sophora flavescens Ait	MT, OMT	0.2% HCl–chloroform	Silica gel G 0.3% CMC–Na		[69]
Su Nv Le gynecologic medicine	OMT	Chloroform–methanol–ammonia (5:6:0.2)	Silica gel G		[70]
Kurainone power medicine	MT	CTAB, SDS	Polyamide 6 membrane		[71]
Boxingkang surongmo	MT	Benzene–acetone–ethyletanoate–ammonia (2:3:4:0.2)	Silica gel G	1–5 μg	[72]
Angelica matrine pill	MT	Toluene–acetone–ethanol–ammonia (10:0:1.5:0.5)	Silica gel G 0.3% CMC–Na	2.4–7.2 μg	[67]
Shenbaiye	MT, OMT	Toluene–ethyletanoate–methanol–H_2O (2:4:2:1)	Silica gel G 0.25% CMC–Na		[73]
Sophora flavescens Ait	MT	Chloroform–methanol–ammonia (5:0.6:0.2)	Silica gel G	2×10^{-6} to 2×10^{-9} g/ml	[74]
Throat spray	MT	Toluene–acetone–ethanol–ammonia (20:20:3:1)	Silica gel G 0.7% CMC–Na	1.8–9.0 μg	[75]
Gynaecologic exterior disinfector	MT	Benzene–acetone–methanol (8:3:0.5)	Silica gel G 3% CMC–Na		[76]

6. Evaluation of the analytical results

The modern HPLC system is a very powerful analytical tool that can provide accurate and precise analytical results, but the major sources of error in quantitation including sampling and sample handling, detector and the choice of chromatographic method should be considered.

Sample collection and preparation are possibly main error in HPLC application. Special precautions need to be taken for solid samples because solid samples may be inhomogenous. In HPLC separation, dried *S. flavescens* root need to be ground carefully to a fine power, and an enough amount samples, generally more than 1 g, was weighed accurately before its extraction. In liquid samples, there may be phase separation or adsorption of a component to container material such as glass or polyethylene. Adsorption is often observed in analyzing macromolecules such as proteins. The extraction efficiency and reproducibility obviously affect sampling precision. In LLE, the composition, content, volume of solvents and even extraction temperature should be carefully taken into account. The selection of extraction solvents for *S. flavescens* root has been discussed in the former chapter. Generally, an organic solvent with chloroform and methanol is employed. The recovery experiments may be helpful in the measurement of extraction efficiency. In a recovery measurement, known amounts of alkaloids are added to an accurately weighed fine power sample of *S. flavescens* root. The mixture of the alkaloids is extracted and analyzed. In Table 4, a recovery from 93.5 to 105% could be observed in the most reported HPLC methods, but Lai et al. [36] reported the recovery was 46.4% for MT, 45.0% for OMT by non-imprinted column. Actually, the yield of the traditional extraction LLE method using above solvent was only about 52% [37], the high recovery reported may indicate some reasons or errors existence in their reports.

The second source of error may be in detector, which may occur due to selective losses of samples onto the stationary phase of column, detector non-linearity and response factors. For the selective losses onto column, spiking and recovery experiments may be helpful in establishing the extent of error introduced by physical losses. Many detectors are linear over only one or two decades of operation. One approach in extending the effective linear range of a detector is high-low injection. In this approach, an accurate dilution of a stock sample solution should be prepared. In Table 4, linearity with more than five decades of alkaloids could be found by high-low injection. The results showed when a ODS-80 Ts reversed-phase column and ECL detector were employed, the peak area was linearly related to sophoridine for the range of 2×10^{-10} to 5×10^{-5} g/ml, 5×10^{-10} to 5×10^{-5} g/ml for matrine, 8×10^{-10} to 7×10^{-5} g/ml for sophoranol, 2×10^{-9} to 6×10^{-5} g/ml for sophocarpine, respectively [47]. Furthermore, measurement of response factors is taken in account and employed in HPLC practice since detectors usually are not equally sensitive to all components of a mixture.

Detectability may be a significant problem in the HPLC application to matrine-type alkaloids. UV detector is commonly used in the HPLC system, but there is few reports on using other kinds of detectors such as fluorescence, electrochemistry or infrared spectrum since these alkaloids may lack of obviously active fluorescent or electrochemical groups. An interesting attempt to apply thin layer flow through ECL detector for HPLC application has been proposed [47]. According to the report, the detection limits were ranged from 3×10^{-11} g/ml for sophoridine, 6×10^{-11} g/ml for matrine, 7×10^{-11} g/ml for sophoranol, 1×10^{-10} g/ml for sophocarpine, respectively, at an S/N of 3. Relative standard deviations were less than 3% for 10 replicate injections for all samples. The chromatographic mode affects the quantitative

accurate. The hydrophobic or hydrophilic character of analytes should be considered before the separation selection. Revised-phase columns are commonly used in the separation of matrine-type alkaloids for their excellent performance.

An external standard method or internal standard method is used in the HPLC quantitative analysis. In external standard, a direct comparison of the detector response of a pure compound to a sample or preparing standards of varying concentration and analyzing them is performed. The key step in the internal standard method is to choose an appropriate internal standard, which has polarity similar to the analyte. Although the internal standard method gives reliable, accurate and precise results. most of the presented references for the HPLC analysis of matrine-type alkaloid show that few internal standard methods have been applied since it is difficult to select a suitable internal standard compound.

7. Conclusion

The root *of S. flavescens* is a widely used traditional Chinese herbal drug. The primary effective components of *S. flavescens* are matrine-type alkaloids, which possess strong biological activities and provide a valuable resource in the treatment of some diseases such as antifebrile, ache, diuretic, tussis, antidote, tumor and so on. It is of important to develop efficient methods for their separation and determination from *S. flavescens* root. HPLC with UV detection is the most widely utilized separation method for the determination matrine-type alkaloids in various matrices. Different sample preparation techniques have been also applied to HPLC or to other chromatographic methods to improve precision, accuracy, and reproducibility. LLE is the most widely employed sample preparation methodologies used for quantitative chromatographic analysis of matrine-type alkaloids in various matrices such as dried *S. flavescens* root, Chinese medicine products and injections. In addition, other separation methods as HPCE, GC or TLC are also proved to be useful in the separation and analysis of matrine-type of *S. flavescens* root.

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