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Review

Separation methods used for *Scutellaria baicalensis* active components

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Abstract

Scutellaria baicalensis Georgi is one of the most widely used traditional Chinese herbal medicines. Its roots have been used for anti-inflammation, anticancer, antiviral and antibacterial infections of the respiratory and the gastrointestinal tract, cleaning away heat, moistening aridity, purging fire, detoxifying toxicosis, reducing the total cholesterol level and decreasing blood pressures. Baicalin, baicalein, wogonin and oroxylin A are its main active components. This review provides an overview of various separation, detection, and identification techniques employed for the quantitative and qualitative determination of these active components. Applications of high-performance liquid chromatography, high-speed counter-current chromatography, thin layer chromatography, capillary electrophoresis, and micellar electrokinetic capillary chromatography to the separation and determination of these active components are described. Examples of identification of these active components and their metabolites in complex matrices by high-performance liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry are also presented. The advantages and limitations of these separation and identification methods are assessed and discussed.

Keywords: Reviews; *Scutellaria baicalensis*; Baicalin

Contents

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1. Introduction

Scutellaria baicalensis (*S. baicalensis*) Georgi (Huangqin in Chinese) is one of the most widely used traditional Chinese herbal medicines, and is officially listed in the Chinese Pharmacopoeia. Its roots have been used for anti-inflammation, anticancer, treating bacterial and viral infections of the respiratory and the gastrointestinal tract, cleaning away heat, moistening aridity, purging fire, detoxifying toxicosis, reducing the total cholesterol level and decreasing blood pressures. This herb also possesses cholagogic, diuretic, and cathartic actions. Some concentrated composite herbal preparations that contain *S. baicalensis* Georgi as a major ingredient in their prescriptions are widely used in oriental countries [1–4]. *S. baicalensis* Georgi contains a variety of flavones, phenylethanoids, amino acids, sterols and essential oils. Its dried roots contain over 30 kinds of flavonoids, such as baicalin, baicalein, wogonin, wogonin 7-*O*-glucuronide, oroxylin A, and oroxylin A 7-*O*-glucuronide. Baicalin, baicalein, wogonin and oroxylin A are the main active components in *S. baicalensis* Georgi [5–8]. Baicalin is the most abundant component, and has anti-allergic [9], anti-inflammatory [10], anti-HIV [11,12], anti-tumor [13–15], antioxidant and free radical scavenging [16,17], and anti-SARS coronavirus effects [18]. Baicalein possesses anti-HIV [11,12], anti-tumor [15], antioxidant and free radical scavenging effects [16,17]. Wogonin has anti-respiratory syncytial virus [19], anti-hepatitis B virus [20], anti-tumor [15], antioxidant and free radical scavenging effects [17]. Oroxylin A has anti-respiratory syncytial virus activity [19].

The quantitative and qualitative determination of pharmaceutical components is critical in all stages of drug discovery. Biological analyses in support of ADME (absorption, distribution, metabolism and excretion), pharmacokinetic, and toxicokinetic studies are crucial in the drug selection and optimization processes [21]. A variety of separation techniques have been employed for the quantitative determination of *S. baicalensis* active components in various matrices. These techniques include high-performance liquid chromatography (HPLC), high-speed counter-current chromatography (HSCCC), thin layer chromatography (TLC), capillary electrophoresis (CE), and micellar electrokinetic capillary chromatography (MEKC). Fig. 1 shows chemical structures of six *S. baicalensis* active components. This review provides an overview of separation, detection, and identification methods employed for these active components in various types of samples.

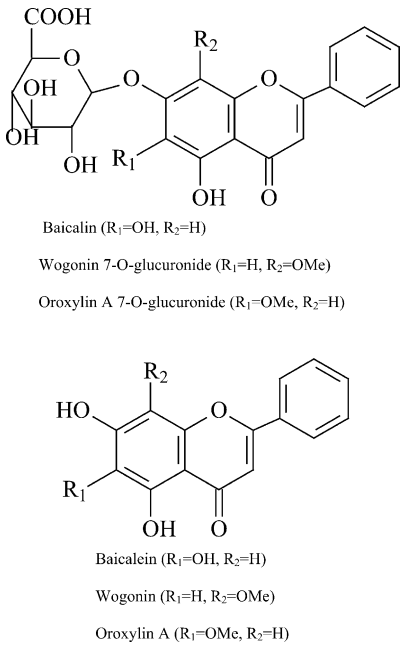

Fig. 1. Chemical structures of six *S. baicalensis* active components.

2. Chromatographic methods

2.1. High-performance liquid chromatography

High-performance liquid chromatographic (HPLC) methods have been widely applied to the separation and determination of *S. baicalensis* Georgi active components in various matrices. Analyses of plant and raw medical material samples are discussed first, followed by medicinal preparations, and then biological fluids (blood and urine) samples.

2.1.1. Analysis of plant and raw medical materials

The active components must be extracted from plant or raw medical material samples prior to analysis by HPLC. Several extraction techniques have been developed [22–24]. Solid-phase extraction (SPE) is a commonly employed sample preparation method for drug compounds in complex matrices. SPE is used to selectively remove interfering matrix components, leading to reduced time required for chromatographic method development and improved assay selectivity, accuracy, and sensitivity. A method combining solid-phase extraction and reversed phase high-performance liquid chromatography was recently developed for simultaneous isolation and determination of flavanes (baicalin, baicalein, chrysin, scutellarein) and some phenolic acids in aerial and underground parts of *S. baicalensis* Georgi [22]. The application of optimized enrichment conditions, elaborated on octadecyl and quaternary amine BakerBond microcolumns, led to the extraction of both groups of analytes with recoveries >95% and variation coefficients <5%. Supercritical fluid extraction (SFE) is another widely used technique for extraction of active components from plant or raw medical material samples, in which supercritical carbon dioxide is often used as an extraction solvent. The use of carbon dioxide has the following advantages: chemically inert, low toxicity, no pollution problem, and shorter concentration time. Under supercritical conditions, the solubility and diffusivity of the analytes in extraction fluid are increased, leading to improved extraction. For the extraction of polar or ionic compounds, organic solvents are added as modifiers or the compounds

are first derivatized to decrease their polarity. SFE was applied to the extraction of baicalin, baicalein and wogonin from *S. baicalensis* [23]. The optimal conditions of SFE were as follows: supercritical carbon dioxide–methanol–water (20:2.1:0.9), 50 °C and 200 bar. The SFE method gave high yields of the three flavanoids in shorter time than ultrasonic or percolation extraction. Pressurized liquid extraction (PLE) with methanol as solvent was proposed for the extraction of baicalein from Scutellariae radix [24]. The comparable performance of PLE with reference to Soxhlet extraction was due to the higher diffusion rate and higher solubility of analyte in the solvent as a result of the higher temperature. At high temperature, the strong solute–matrix interaction in the plant materials caused by van der Waals forces, hydrogen bonding and dipole attractions between solute molecules and active sites on the matrix were disrupted. To reduce the use of organic solvent, pressurized hot water extraction (PHWE) was developed for the extraction of baicalein from Scutellariae radix [24]. Ethanol as organic modifier was added into the liquid used in PHWE. PHWE was carried out dynamically at a flow-rate of 1 ml min^{-1}, temperature between 95 and 140 °C, an applied pressure of 10–20 bar and extraction time of 40 min. Although baicalein was insoluble in water, the results showed that water with a small proportion (20%) of ethanol at a temperature below its boiling point and a small applied pressure was able to extract an equivalent amount of baicalein from medicinal plant compared with Soxhlet extraction with aqueous organic solvent. The results obtained by PHWE were in agreement with those using PLE with methanol as the extraction solvent.

Several methods have been proposed for the determination of *S. baicalensis* Georgi active components in plant or raw medical material samples by HPLC [25–29]. Baicalin was determined by HPLC on a Hypersil C_{18} column (25 cm × 4.6 mm i.d.) with methanol/0.04% H_3PO_4 (23:27) as mobile phase (1 ml min^{-1}) and detection at 280 nm [25]. The method was applied to the determination of baicalin in the raw medical materials and two different processing drugs of *S. baicalensis*, and the contents of baicalin were 6.8, 6.0 and 6.73%, respectively. Although the method was simple, only one component was determined in a single run. In another method, two components were determined by HPLC in a single run on an YMC-Pack ODS A-132 column (15 cm × 6 mm i.d.) with methanol/0.1M phosphate buffer as mobile phase (1:1 for baicalin and wogonin, or 17:8 for baicalein and wogonin 7-*O*-glucuronide) and detection at 270 nm [26]. The method was applied to the comparative study of the concentrations of four flavonoids in two Scutellaria species (S.). The concentrations of baicalin, baicalein, wogonin and wogonin 7-*O*-glucuronide were 12.77, 3.53, 0.93 and 3.56 and 14.40, 3.08, 0.97 and 2.99%, respectively in *S. planipes* and *S. baicalensis*. Actually, more components could be determined simultaneously by HPLC [27–29]. The three components, baicalin, baicalein and wogonin in Scutellariae radix were determined by HPLC on a 10 μm ODS Hypersil column (25 cm × 4.6 mm i.d.) with gradient elution of acetonitrile and 0.1 M H_3PO_4 as mobile phase and detection at 280 nm [27]. The six main bioactive components, baicalein, baicalin, wogonin, wogonin glucuronide, oroxylin-A and oroxylin-A glucuronide in Scutellariae radix could be determined simultaneously by ion-pair high-performance liquid chromatography on a stainless-steel column (15 cm × 4 mm i.d.) packed with TSK gel LS-410 (5 μm) with aqueous 32% acetonitrile, containing 5 mM tetrapentylammonium bromide, as mobile phase, adjusted to pH 4 with H_3PO_4 [28]. Oroxylin-A and its glucuronide were separated for the first time. The method was one of the best methods for the simultaneous determination of main active components in *S. baicalensis* Georgi. In another study, flavonoid constituents of the roots of *S. baicalensis* Georgi were determined by HPLC on a column (20 cm × 6 mm) of Develosil ODS-5 at 50 °C, with 274 nm detection and tetrahydrofuran–dioxan– methanol–acetic acid–5% H_3PO_4–H_2O (145:125:50:20:2:322) or tetrahydrofuran–acetic acid–5% H_3PO_4–H_2O (95:10:1:444) as mobile phase [29]. By a combination of two mobile phases, total 11 flavonoids were separated in two runs. In addition, the content of the main flavonoids in the roots of *S. baicalensis* Georgi cultivated in Central Europe was evaluated by an HPLC method with gradient of acetonitrile in mobile phase [30]. The main components of the roots were baicalin (8.12% of dry root mass) and wogonin glucuronide (2.52%). The content of flavonoids was comparable with the content in plants cultivated in natural localities.

2.1.2. Analysis of medicinal preparations

Because baicalin is the main bioactive constituent of *S. baicalensis* Georgi, many reports have been published on the determination of baicalin by HPLC methods in different traditional Chinese medicinal preparations, such as No. 1 cold capsulae [31], Qingxiewan [32], Yizhangxiao capsule [33], heat-clearing decoction [34], Fructus Sophorae pills [35], compound Houttuynia cordata granules [36], Qinghouyan granules [37], twelve baicalin-containing preparations such as pills, tablets and injection solutions [38], Biyankang tablets [39], and Huanglian Shangqing Wan [40]. HPLC methods have been also applied to the determination of baicalin and chlorogenic acid in Yinhuang oral liquid [41], baicalin and berberine in Wanshi Niuhuang Qingxin Wan [42], baicalin and puerarin in medicinal preparations [43], baicalin, sennoside A and glycyrrhizin in Niuhuangjiedupian [44], baicalin, paeoniflorin and ferulic acid in Dang-Guei-San [45]. In these methods, reversed-phase columns such as C_{18} are the most frequently used. Mobile phases usually consist of a combination of an acidic buffer (or an aqueous phase with an acid H_3PO_4 or acetic acid as additive) and an organic solvent component, usually methanol or acetonitrile. The use of an acid modifier is to suppress ionization of the acidic groups and interactions of these groups with residual traces of metals in the stationary phase that are detrimental to peak shape.

Several bioactive components from *S. baicalensis* Georgi could be determined simultaneously in medicinal

preparations by HPLC [46,47]. Two components, baicalin and baicalein in processed Scutellariae radix and its pharmaceutical preparations (e.g. slices for infusion) were determined by HPLC on a column (15 cm × 6 mm i.d.) of ODS with THF–dioxane–methanol–*n*-propanol–acetic acid–5% H_3PO_4–H_2O (145:125:50:20:20:2:638) as mobile phase (1.5 ml min^{-1}) and detection at 274 nm [46]. Three components, baicalin, baicalein and wogonin in traditional decoctions and commercial extracts of Scutellariae radix were determined by HPLC with ethyl paraben as internal standard [47]. The analytes were separated on a 5 μm Inertsil ODS-2 column (25 cm × 4.6 mm i.d.) with acetonitrile–0.005% phosphoric acid (9:16) as mobile phase (1 ml min^{-1}) and detection at 270 nm. The method was used to compare the absorption of baicalin, baicalein, wogonoside and wogonin between traditional decoction and a commercial powder preparation of Scutellariae radix [48]. The bioavailability of wogonin/wogonoside was about two times when compared with that of baicalein/baicalin from either traditional decoction or the commercial preparation. The flavone bioavailability from commercial preparation was significantly lower by 44.2 ± 0.1% for baicalin/baicalein and by 42.3 ± 0.1% for wogonoside/wogonin than those from traditional decoction.

For compound medicinal preparations, simultaneous determination of many bioactive constituents is needed for quality control. The six components, paeoniflorin, cinnamic acid, baicalin, baicalein, wogonin and glycyrrhizin in medicinal preparation Chai-Hu-Kuei-Chih-Tang were determined by HPLC [49]. Commercial powdered sample was ultrasonicated with 70% methanol and filtered. The filtrate was analysed on a Cosmosil 5 C_{18}-AR column (25 cm × 4.6 mm i.d.) with linear gradient elution of acetonitrile/aqueous 20 mM H_3PO_4 as mobile phase and UV detection. The seven constituents, gentiopicroside, mangiferin, palmatine, berberine, baicalin, wogonin and glycyrrhizin in the traditional Chinese medicinal preparation Sann-Joong-Kuey-Jian-Tang could also be determined by HPLC [50]. Decoctions of the crude drug and concentrated preparations were diluted with aqueous 70% methanol, mixed with *n*-propylparaben as internal standard and analysed on a Cosmosil 5C_{18}-MS column (15 cm × 4.6 mm i.d.) with gradient elution of 0.03% H_3PO_4/acetonitrile as mobile phase and detection at 254 nm. The chromatogram is shown in Fig. 2. In another method, twelve constituents, baicalin, baicalein, wogonin, wogonin 7-*O*-glucuronide, oroxylin A 7-*O*-glucuronide, sennoside A, sennoside B, emodin, aloe–emodin, glycyrrhizin, ferulic acid and caffeic acid in a Chinese herbal preparation I-Tzu-Tang were determined simultaneously by HPLC on a 5 μm reversed-phase Cosmosil 5C_{18}-AR column (25 cm × 4.6 mm i.d.) eluted at a flow rate of 0.8 ml min^{-1} with a linear solvent gradient of tetrabutylammonium bromide/phosphorate buffer/methonal/acetonitrile as mobile phase and diode array detection at 254 nm [51]. The chromatogram is shown in Fig. 3. Furthermore, a method was developed for the simultaneous determination of 14 constituents, baicalin, baicalein,

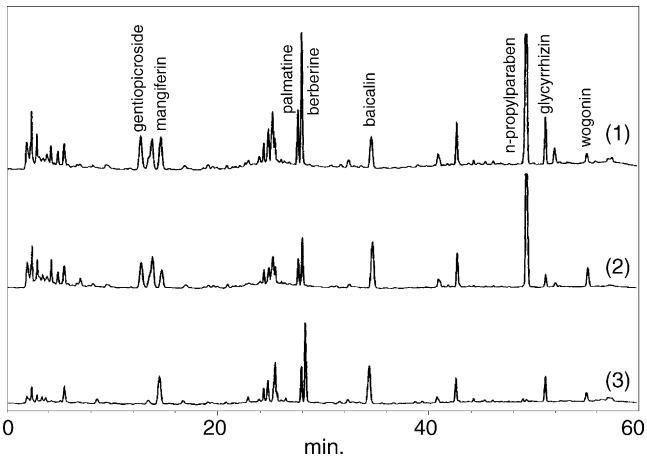

Fig. 2. Chromatogram of the marker substances gentiopicroside, mangiferin, palmatine, berberine, baicalin, glycyrrhizin and wogonin in Sann-Joong-Kuey-Jian-Tang. Column, Cosmosil 5C_{18}-MS, 150 mm × 4.6 mm i.d.; guard column, Cosmosil 5C_{18}-AR, 50 mm × 4.6 mm i.d., mobile phase, 0.03% phosphoric acid–acetonitrile (0 min, 90:19; 10 min, 87:13; 17–27 min, 77:23; 40 min, 62:38; 50 min, 55:45); flow-rate, 1.0 ml min^{-1}. (1) Standard decoction; (2) commercial preparation; (3) standard decoction without *Gentianae scabrae* radix. Reprinted from [50] with permission from Elsevier.

wogonin, wogonoside, oroxylin-A glucoside, orxylin-A, paconiflorin, glycyrrhizic acid, glycyrrhetinic acid, liquiritin, isoliquirition, liquiritigenin, isoliquiritigenin and ononin in the traditional Chinese medicinal preparation Huangqin-Tang by HPLC [52]. Huangqin-Tang samples were mixed and boiled with water, centrifuged then concentrated and analysed on a 5 μm Wakosil II 5C_{18}-AR column (15 cm × 4.6 mm i.d.), methanol/acetic acid and H_2O/acetic acid as mobile phase with gradient elution and diode array detection from 200 to 400 nm.

2.1.3. Analysis of biological fluids

Only a few reports have been published for the determination of the active components from *S. baicalensis* Georgi in biological fluids (blood and urine) samples. Baicalin in rabbits' plasma was determined by a reversed-phase HPLC [53,54]. The sample (0.2 ml) was vortex-mixed with 4-nitrobenzoic acid (internal standard) and acetonitrile. The mixture was shaken ultrasonically and centrifuged. The supernatant was analysed on a 5 μm Dimonsil C_{18} column (20 cm × 4.6 mm i.d.) with methanol–H_2O–H_3PO_4 (25:25:0.1) as mobile phase (1 ml min^{-1}) and detection at 277 nm. In another study, the metabolites of baicalein in human urine were determined by HPLC on a 4 μm ODS column (15 cm × 3.9 mm i.d.) with acetonitrile–0.08 M formic acid (1:4) as mobile phase (1 ml min^{-1}) and photodiode array detection at 315.6 nm [55]. Three main metabolites of baicalein were identified and found to be in agreement with previous studies. In addition, the flavonoids in human urine after administration of a medicinal preparation Shosaiko-to were determined by HPLC [56–58]. An ODS or a Chiracel column (25 cm × 4.6 mm i.d.) was used for nonchiral or chiral analysis, respectively. Mobile phases

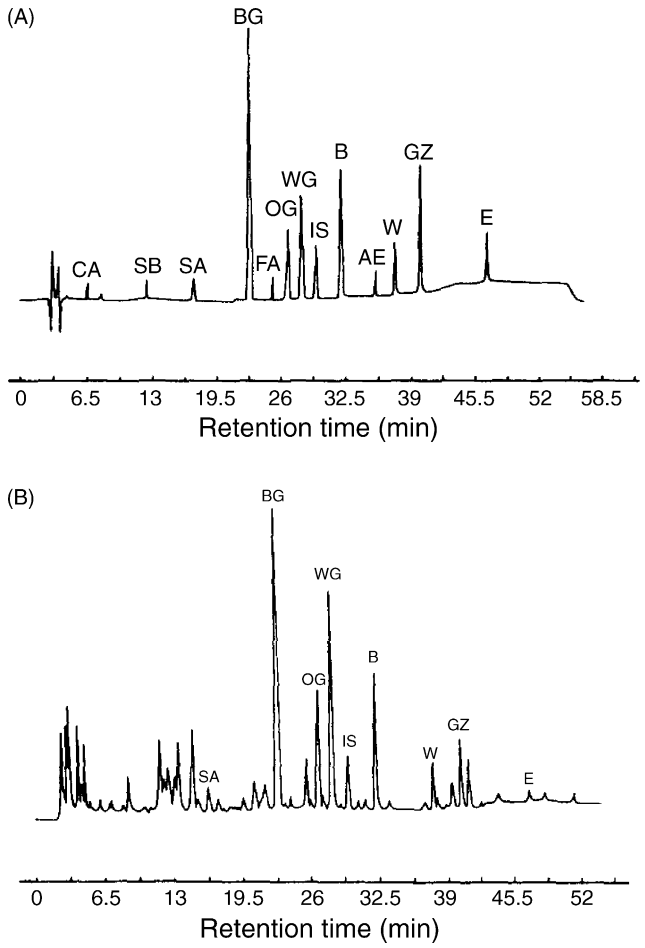

Fig. 3. High-performance liquid chromatograms of the standard solution (A) and I-Tzu-Tang (B). AE: aloe–emodin, B: baicalein, BG: baicalin, CA: caffeic acid, E: emodin, FA: ferulic acid, GZ: glycyrrhizin, IS: internal standard (tetralin), OG: oroxylin A 7-*O*-glucuronide, SA: sennoside A, SB: sennoside B, W: wogonin, WG: wogonin 7-*O*-glucuronide. Reprinted from [51] with permission from Elsevier.

used included acetonitrile/H_2O/acetic acid for flavonoids, isopropanol/H_2O/acetic acid for racemic liquiritin, or *n*-hexane/ethanol/acetic acid for chiral analysis. Detection was at 279, 275, and 295 nm for davidigenin and liquiritin, wogonin and oroxylin A and baicalein, and for dihydrowogonin and dihydrooroxylin A, respectively. Four flavonoids, liquiritigenin, baicalein, wogonin and oroxylin A, were found both in the urine and Shosaiko-to. The glycosides in Shosaiko-to were absorbed after microflora hydrolysis. Two flavanones, S-dihydrowogonin and S-dihydrooroxylin A, were identified as the metabolites of wogonin and oroxylin A, respectively.

Although UV detection is still the most widely used, electrochemical detection is employed for improved specificity and sensitivity. Baicalin and baicalein in rat plasma were determined by HPLC with electrochemical detection [59]. Baicalin was extracted from plasma by SPE with 2,3-dihydroxynaphthalene as internal standard; baicalein was extracted at low or high concentration by SPE or liquid–liquid extraction with quercetin as internal standard. The analyte was determined on a stainless steel Nova-Pak column (15 cm × 3.5 mm i.d.) of octadecylsilane C_{18} (4 μm), operated at 50 °C, with a mobile phase (1.2 ml min^{-1}) of 0.1 M H_3PO_4–acetic acid–THF (800:80:57) for baicalin or a mobile phase (0.8 ml min^{-1}) of 0.2 M H_3PO_4–methanol–THF (500:250:34) for baicalein. Following separation baicalin and baicalein were oxidized at a vitreous C electrode to permit selective electrochemical detection. The method was more sensitive than the HPLC method with UV detection.

2.2. High-speed counter-current chromatography

High-speed counter-current chromatography (HSCCC) is a unique liquid–liquid partition chromatography that uses no solid support matrix. Therefore, it eliminates irreversible adsorptive loss of samples onto the solid support matrix used in the conventional chromatographic column. The method has been successfully applied to the analysis and separation of various natural products [60–62]. In our laboratory, HSCCC has been applied to the isolation and purification of several bioactive compounds from the Chinese medical plants, such as tanshinones and salvianolic acid B in *Salvia miltiorrhiza* Bunge [63,64], salidroside in *Rhodiola sachalinensis* A. Bor [65], shikonin in *Lithospermum erythrorhizon* Sieb. et Zucc. [66], chlorogenic acid in *Flos Lonicerae* [67], glycyrrhizin in *Glycyrrhiza uralensis* Fisch [68].

Baicalin was separated and purified from *S. baicalensis* Georgi by HSCCC [4]. Crude baicalin was obtained by extraction with methanol–water (70:30) from *S. baicalensis* Georgi. The separation was performed in two steps with a two-phase solvent system composed of *n*-butanol–water (1:1), in which the lower phase was used as the mobile phase at a flow-rate of 1.0 ml min^{-1} in the head-to-tail elution mode. The HSCCC chromatogram is shown in Fig. 4. A total of 37.0 mg of baicalin at 96.5% purity was yielded from 200 mg of the crude baicalin (containing 21.6% baicalin) with 86.0% recovery. The simultaneous separation and purification of several active components in *S. baicalensis* Georgi by HSCCC in a single run is being investigated in our laboratory.

2.3. Thin layer chromatography

Thin layer chromatography (TLC) is a quick, convenient, and inexpensive technique widely employed for pharmaceutical analyses. The main advantage of TLC is its ability to assay many samples in parallel on a single TLC or high-performance TLC plate, leading to a large increase in sample throughput compared with the column techniques such as HPLC. However, it also possesses several limitations including lower separation efficiency and capacity compared with the column separation.

A method was developed for the separation of baicalin, baicalein, wogonin, and oroxylin A in Scutellariae radix by high-performance TLC on phenyldimethylethoxysilane-treated silica plates with methanol-phosphate buffer (pH 6.2) as mobile phase [69]. Quantitative determination of the

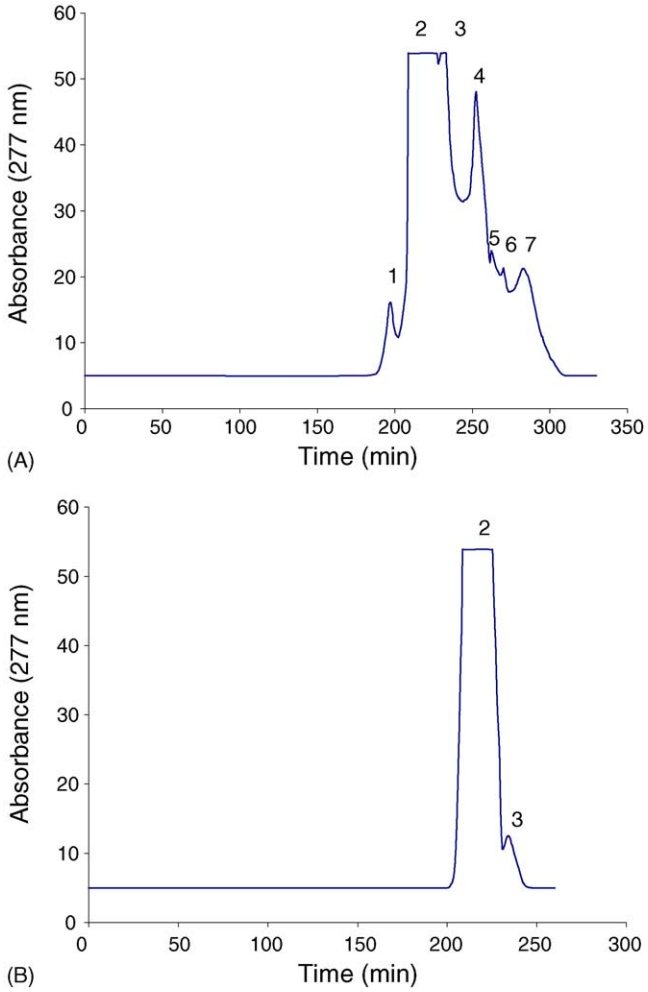

Fig. 4. Chromatogram of the crude baicalin extracted from *S. baicalensis* Georgi by HSCCC separation. Curve 2: baicalin. Conditions: column, multilayer coil of 1.6 mm i.d. PTFE tube with a total capacity of 342 ml; rotary speed, 900 rpm; solvent system, *n*-butanol–water (1:1, v/v); mobile phase, the lower phase; flow-rate, 1.0 ml min^{-1}; detection, 277 nm; sample size, 200 mg; injection volume, 5 ml; retention of the stationary phase, 41.8%; (A) the first separation; (B) the second separation. Reprinted from [4] with permission from Elsevier.

flavonoids was conducted with a dual-wavelength flying-spot scanner at 280 nm.

Thin layer chromatography has been widely applied to separation and determination of baicalin in medicinal preparations, such as, Qingkailing injection solution [70], Xiaochaihutang oral liquid [71], injections and tablets [72], fast-acting anti-diarrhoeal tablet [73], and antibiotics [74]. TLC methods have been also applied to the separation of baicalin and berberine sulfate in HHK injection solution [75], baicalin and emodin in Fangfeng Tongsheng pills [76], and baicalin and chlorogenic acid in Yinhuang tablets and injection solutions [77,78] and in Tongkangtai oral-medication solution [79].

Baicalein and wogonin in the Korean crude drug preparation Soshiho Tang were separated by TLC on Silica gel 60 with benzene–ethyl acetate (1:1) as mobile phase and detection with 20% H_2SO_4 as spray reagent and heating at 110 °C [80]. In another method, baicalin, ibuprofen, rhein and berberine hydrochloride in Sanhuang tablets were determined by TLC scanning method [81]. The powdered sample was extracted by Soxhlet with 95% ethanol. The diluted extract was subjected to TLC on silica gel with benzene–ethyl acetate–acetic acid (150:50:3) as mobile phase and detection by scanning at 430 and 425 nm for determining ibuprofen and rhein, respectively; this TLC plate was then heated at 80 °C for 10 min and developed with $CHCl_3$–ethyl acetate–methanol–formic acid (7:3:1:1) for separating berberine hydrochloride and baicalin. Detection was at 420 and 280 nm for berberine hydrochloride and baicalin, respectively.

3. Electromigration methods

3.1. Capillary electrophoresis

Capillary electrophoresis (CE) is based on different mobilities of ions in electric field, and is applied to the analysis of charged species. CE is a separation technique of high efficiency with low sample and solvent consumption. It has been widely used for separation of both large and small drug molecules. This method, however, has low sensitivity and low reproducibility compared with HPLC methods [21,82].

CE with electrochemical detection operated in amperometric mode can provide higher sensitivity and selectivity than CE with UV detection. Baicalin and baicalein were determined by capillary electrophoresis with electrochemical detection [83]. The two analytes could be well separated within 8 min in a capillary (40 cm × 25 μm i.d.) operated at a separation voltage of 12 kV, with 100 mM borate buffer (pH 9.0) as running buffer. The working electrode was a 300 μm diameter carbon disc electrode at a potential of 0.90 V. The method was employed for the differentiation of Scutellariae radix from Astragali radix. Scutellariae radix contains a great deal of baicalin and baicalein that are not present in Astragali radix, so both these crude drugs can be differentiated by determining their baicalin and baicalein contents. A similar method was applied to simultaneous determination of baicalein, baicalin and quercetin in Scutellariae radix and its preparations [84]. The similar method was also used to the determination of baicalein, baicalin, and chlorogenic acid in traditional medicinal preparation Yinhuang oral liquid [85].

CE with UV detection, however, is more widely employed than CE with electrochemical detection. Baicalin in prescriptions containing *S. baicalensis* Georgi was determined by high-performance capillary electrophoresis [86]. The sample solution with *p*-nitrobenzoic acid as internal standard was analysed on an uncoated quartz column (39.5 cm × 50 μm; effective length 34.8 cm), operated at an applied voltage of 17 kV, with 40 mM borax buffer (pH 9) as running buffer and detection at 285 nm. In another method, two constituents, baicalin and chlorogenic acid in Yinhuang granules were determined by CE [87]. The sample solution with

p-nitrobenzoic acid as internal standard was analysed on a bare fused-silica capillary (47 cm × 50 μm i.d.; effective length 40 cm), operated at an applied voltage of 25 kV, with 25 mM borax at pH 8.5 as running buffer and detection at 310 nm. A method was developed for the simultaneous determination of three constituents, baicalin, chlorogenic acid and caffeic acid in seven traditional Chinese medicinal preparations containing Honeysuckle flower and/or Scutellariae radix by capillary zone electrophoresis [88]. The analytes were separated successfully within 3.5 min using 10 mM borate buffer (pH 8.6). Furthermore, the six bioactive constituents in San-Huang-Hsieh-Hsin-Tang were determined by CE [89]. The sample solution with methyltriphenylphosphonium iodide as internal standard was injected hydrostatically for 5 s into a fused-silica capillary (80 cm × 75 μm i.d.; 72.5 cm to detector) for separation at 25 kV and detection at 254 nm. The run buffer contained 50 mM sodium cholate, 50 mM NaH_2PO_4, 4.25 mM sodium borate and 40% acetonitrile. Separation of berberine, palmatine, baicalin, serinoside B, emodin, and sennoside A was achieved in 20 min. A similar method was applied to the determination of these six bioactive components in Hsiao-Cheng-Chi-Tang [90].

3.2. Micellar electrokinetic capillary chromatography

Micellar electrokinetic capillary chromatography (MEKC) is based on partition between bulk solution and micelle moving in opposite direction to analyte, and is used to determine neutrals. MEKC was employed as alternatives to HPLC for separations of some special samples such as enantiomeric mixtures and very polar compounds [21,82]. MEKC with UV detection has been widely employed for *S. baicalensis* active components.

Baicalein, baicalin, wogonin and acteoside in wild and cultivated *S. baicalensis* roots were determined by MEKC [91]. The analysis of plant extracts was carried out on a fused silica capillary column (82 cm × 50 μm i.d.; 70 cm to detection window) at 25 °C with buffer (pH 8.73) consisting of 30 mM SDS, 10 mM NaH_2PO_4 and 15 mM $Na_2B_4O_7$, siphon injection (25 mm, 30 s), an applied voltage of 30 kV and UV detection at 275 or 350 nm. The method can be used for the quality control of Scutellariae radix in a shorter analysis period than HPLC. In another study, the six flavonoids, baicalin, baicalein, wogonin 7-*O*-glucuronide, wogonin, oroxylin A 7-*O*-glucuronide, and oroxylin A in Scutellariae radix were determined by MEKC [92]. The extract with salicylic acid as internal standard was analysed on a fused-silica capillary (1 m × 75 μm i.d.; 95.4 cm to detector) at 20 °C with an electrolyte of pH 9.7 containing 20 mM SDS, 10 mM sodium dihydrogen phosphate and 12.5 mM sodium borate, an applied voltage of 30 kV and detection at 275 nm. A similar method was used for the simultaneous determination of these six flavonoids in Scutellariae radix by MEKC with UV detection at 254 nm [93].

For medicinal preparations, baicalin, chlorogenic acid and forsythin in Shuanghuanglian oral liquid were determined by MEKC on a bare fused-silica capillary (60 cm × 50 μm i.d.) operated at an applied voltage of 15 kV, using borate buffer, SDS and acetonitrile as background electrolyte and UV detection [94]. The three compounds were completely separated within 15 min. In another method, four constituents, baicalein, baicalin, wogonin and wogonin 7-*O*-glucuronide in traditional Chinese medicinal preparations were determined by MEKC [95]. The sample solution with salicylic acid as internal standard was analysed on a fused-silica capillary (100 cm × 75 μm i.d.; 95.4 cm to detection window) at 20 °C with an applied voltage of 30 kV, an electrolyte of 20 mM SDS/10 mM sodium dihydrogen phosphate/12.5 mM sodium borate buffer and detection at 275 nm. Injection was performed at 3 psi for 1 s. A method was developed for the simultaneous determination of ten constituents, baicalin, wogonin 7-*O*-glucuronide, oroxylin A 7-*O*-glucuronide, baicalein, wogonin, oroxylin A, berberine, palmatine, coptisine and epiberberine in the scute-coptis herb couple by MEKC with a photodiode array detector operating at 270 nm [96]. The carrier composed of a buffer solution (5 mM sodium berate, 15 mM sodium dihydrogenphosphate and 50 mM sodium cholate) and acetonitrile (3:2) was found to be the most suitable electrolyte for this separation, whereby the contents of these compounds in the herb couple and herb couple containing Chinese herbal preparations could be determined within 30 min. The electropherogram is shown in Fig. 5. Furthermore, the twelve constituents, baicalin, baicalein, wogonin, wogonin 7-*O*-glucuronide, oroxylin A 7-*O*-glucuronide, sennoside A, sennoside B, emodin, aloe–emodin, glycyrrhizin, ferulic acid and caffeic acid in a Chinese herbal preparation I-Tzu-Tang could be determined simultaneously by MEKC on a fused-silica capillary tube (70 cm × 75 μm i.d.; 62.5 cm to detection window) at 30 °C with an applied voltage of 20 kV, an electrolyte solution consisting of 18 mM SDS, 2 mM sodium cholate, 12.5 mM $Na_2B_4O_7$ and 10 mM NaH_2PO_4 and detection at 254 nm [51]. Injection was performed at 2 psi for 1 s. The electropherogram is shown in Fig. 6. The contents of these components in an unpretreated I-Tzu-Tang extract could be determined in a shorter time by MEKC (14 min) than by HPLC (50 min) (Fig. 3). Moreover, all 11 components of I-Tzu-Tang could be determined by MEKC (Fig. 6B), but only eight components could be determined by HPLC owing to serious interference from some impurities (Fig. 3B). Compared with the HPLC method, the MEKC method is more attractive, especially owing to its shorter running time, and therefore should be useful for the determination of large numbers of samples and for quality control in pharmaceutical plants.

4. Hyphenation procedures

4.1. High-performance liquid chromatography–mass spectrometry

High-performance liquid chromatography–mass spectrometry is applied for the separation and identification

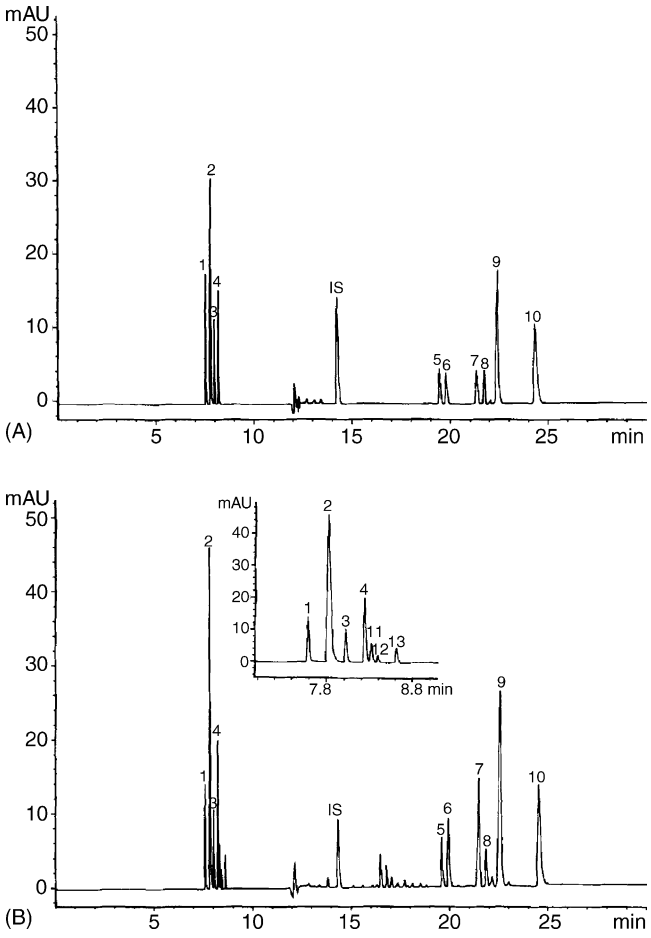

Fig. 5. Electropherograms of the standard solution (A) and the scute-coptis herb couple (B) with internal standard (IS). 1, coptisine; 2, berberine; 3, epiberberine; 4, palmatine; IS, umbelliferone; 5, oroxylin A; 6, wogonin; 7, wogonin 7-*O*-glucuronide; 8, oroxylin A 7-*O*-glucuronide; 9, baicalin; 10, baicalein. Reprinted from [96] with permission from Elsevier.

of unknown mixtures in simple or complex matrices. The main advantages of HPLC–MS are its high speed and sensitivity compared with other hyphenated identification techniques such as HPLC–nuclear magnetic resonance (NMR) and HPLC–infrared. In most situations, HPLC–MS/MS or HPLC–$(MS)^n$ is preferred over HPLC–MS for the structure elucidation of unknown mixtures, because multistage MS can provide additional information on fragmented ions, facilitating structural assignments [21].

The active components in the traditional Chinese medicine Xietin decoction were separated and identified of by liquid chromatography–mass spectrometry [97]. The analytes were separated on an YWC ODS-AQ column (5 cm × 2 mm i.d.) with linear gradient elution of H_2O/ammonium acetate/formic acid/methanol as mobile phase and atmospheric-pressure-ionization tandem MS detection operating in multiple reaction monitoring. Nine active ingredients, rhein, aloe–emodin, physcion, baicalin, wogonoside, berberine, coptine, jatrorrhizine and palmatine were simultaneously determined.

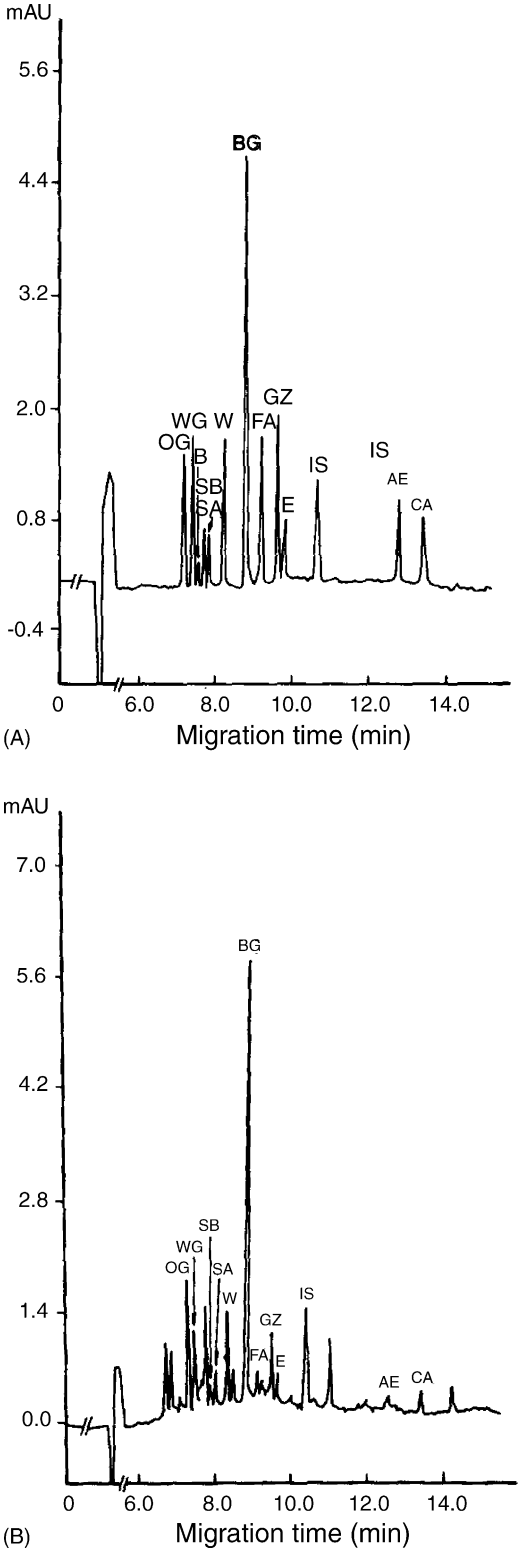

Fig. 6. Electropherograms of the standard solution (A) and I-Tzu-Tang (B). IS: internal standard (salicylic acid), other symbols as in Fig. 3. Reprinted from [51] with permission from Elsevier.

Wogonin in rat plasma was determined by liquid chromatography-tandem mass spectrometry [98]. The analyte with daidzein as internal standard was determined on a 5 μm Diamonsil C_{18} column (25 cm × 4.6 mm i.d.) with acetonitrile–H_2O–formic acid (80:20:1) as mobile phase (0.8 ml min^{-1}) and tandem positive-ion atmospheric pressure chemical ionization MS detection operated in selected-reaction monitoring mode. In another method, wogonin and its major metabolite in rat plasma was determined by liquid chromatography-tandem mass spectrometry [99]. Only one conjugated metabolite with glucuronic acid was identified by chromatographic and electrospray multi-stage MS assay (Fig. 7). A derivatization reaction with 2-chlorethanol further demonstrated that the metabolite was wogonin 7β-D-glucuronide, not wogonin 5β-D-glucuronide. Other conjugated metabolites, e.g., sulfates and glucosides, were not detected. The plasma concentration of free wogonin was determined using atmospheric pressure chemical ionization source in the selected reaction monitoring (SRM) mode (Fig. 8). Incubation of the plasma samples with β-glucuronidase allows the quantitation of wogonin 7β-D-glucuronide.

4.2. Gas chromatography–mass spectrometry

Because of the high polarity, low volatility and poor thermal stability, gas chromatography was not widely employed for the determination of baicalin, baicalein or wogonin. As a result, few gas chromatographic methods were developed and reported in the literature. These active compounds in the crude extracts of *S. baicalensis* Georgi were identified by gas chromatography–mass spectrometry [23]. A 30 m × 0.25 mm HP-5MS (crosslinked 5% diphenyl–95% dimethylpolysiloxane) capillary column was used (0.25 μm film thickness) with helium as carrier gas. The molecular ion peaks of wogonin and baicalein were at *m/z* 284 and 270, respectively. As to baicalin, its molecular ion peak could not be observed at *m/z* 446. All crude extracts showed the similar patterns of total ion chromatogram. By comparison of MS fragments with library search software, wogonin was identified. But at the retention time of 20.03 min, fragments of *m/z* 270 had a relatively low intensity compared with those of *m/z* 284, 269, 139 and 241. By comparison with the peak intensities of the library database, these peaks may be shown to represent the combinations of many constituents, such as baicalin, baicalein and oroxylin A. Therefore, optimal conditions for separation of the coexisting baicalein and baicalin by GC–MS require further investigation.

5. Quantitation and validation

Often minor reagent substitutions, changes in laboratory temperature, or lot-to-lot variations in extraction cartridges and analytical columns affect analytical results. The use of internal standard method can compensate for sample losses occurring during extraction, clean-up and final analysis and for signal changes caused by variation in experimental conditions. An ideal internal standard should resemble the analyte as closely as possible in terms of chemical and physical properties and this requirement is fulfilled when a structural analogue is selected as an internal standard.

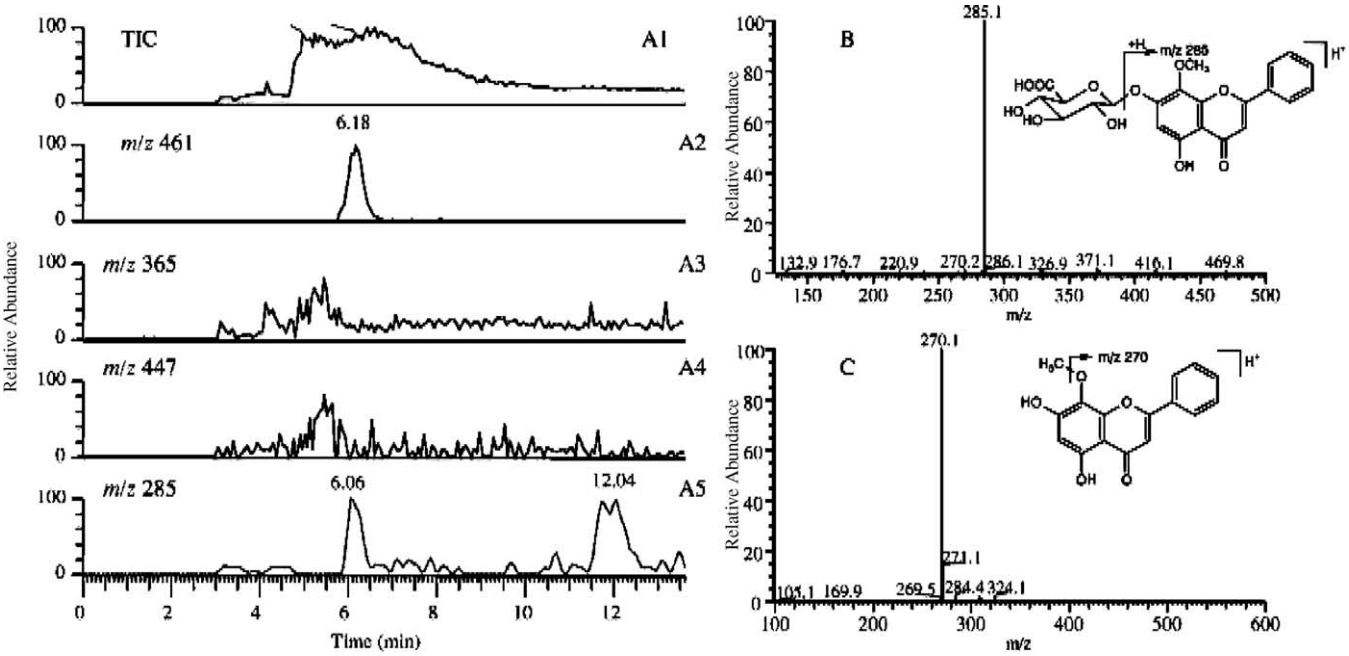

Fig. 7. LC–MS^n analysis of wogonin and its conjugated metabolites in plasma after an administration of 5 mg kg^{-1} wogonin to a Wistar rat. (A) Total ion current and selected ion monitoring chromatograms (A2, wogonin glucuronide; A3, wogonin sulfate; A4, wogonin glucoside; A5, free wogonin); (B) full scan MS–MS spectrum of peak at 6.18 min; (C) full scan MS^3 spectrum of peak at 6.18 min. Reprinted from [99] with permission from Elsevier.

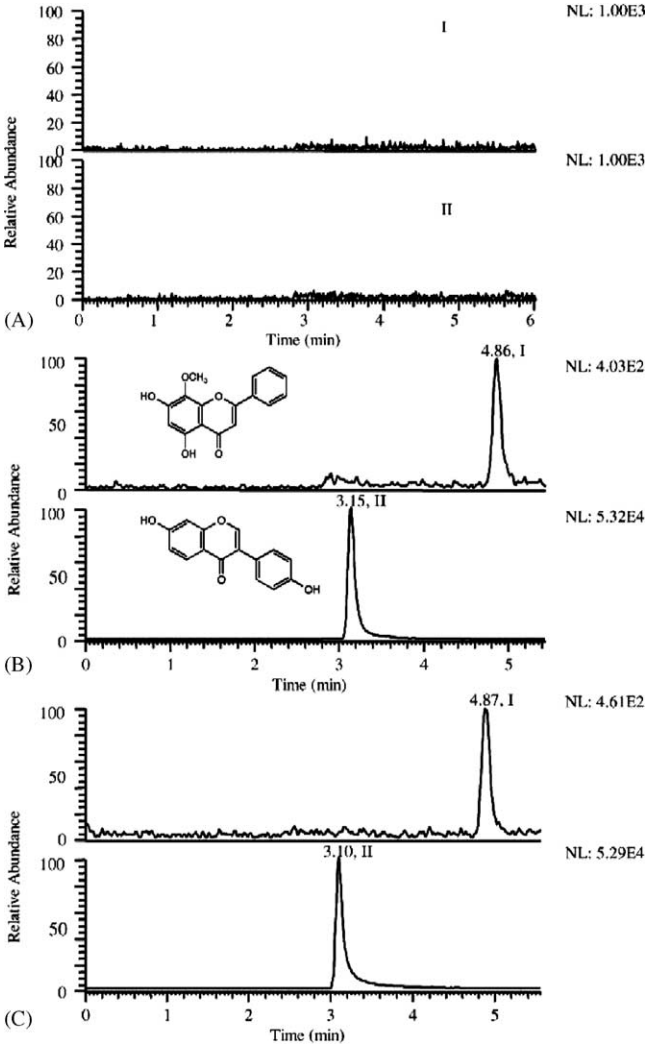

Fig. 8. Representative SRM chromatograms of: (A) a blank rat plasma sample; (B) a blank plasma sample spiked with 2.0 ng ml^{-1} wogonin and the internal standard daidzein (IS, 1000 ng ml^{-1}); (C) plasma sample from a Wistar rat 0.5 h after an oral administration of wogonin (5 mg kg^{-1}). Peak I, wogonin; peak II, IS. Reprinted from [99] with permission from Elsevier.

Good science and effective medical care demand inexpensive validated methods with high throughput which are capable of simultaneously analyzing multiple drugs in various matrices. The analytical methodology should be validated in terms of precision, accuracy, limit of detection, limit of quantitation, specificity, linearity and range, ruggedness and robustness [100].

In a method for the HPLC-UV determination of baicalin, baicalein and wogonin in Scutellariae radix, ethyl paraben was used as internal standard. The linearity ranges were 3.1–100, 2.5–80 and 2.5–80 μg ml^{-1}, respectively. The determination and detection limits were 2.5–3.1 and 0.2–0.3 μg ml^{-1}, respectively. The recoveries were 87.8–110% with the intra- and inter-run R.S.D. of 5.3% [47]. In another HPLC-UV method for the determination of baicalin in rabbit serum, *p*-nitrobenzoic acid was used as internal standard. The standard curve was linear from 0.5 to 200.0 mg l^{-1} with a correlation coefficient of 0.9994. The recovery was 98.2–101.8%. The intra- and inter-day R.S.D.s were 3.7–5.4 and 5.3–7.2%, respectively [54]. When baicalin and baicalein in rat plasma were determined by HPLC with electrochemical detection, the linearity ranges were 5–2000 and 2–5000 ng ml^{-1}, respectively. The detection limits were 5 and 2 ng ml^{-1}, respectively and corresponding coefficients of variation were 4.6–8.6% and 0.6–8.8% [59]. The method was more sensitive than the HPLC method with UV detection [47].

For determination of baicalin, ibuprofen, rhein and berberine hydrochloride in Sanhuang tablets by TLC scanning method, the calibration graphs were rectilinear for up to 2, 1.2, 5 and 10 μg for ibuprofen, rhein, berberine hydrochloride and baicalin, respectively. The recoveries averaged >94% with the coefficients of variation of <4% [81].

When baicalin, baicalein and quercetin in Scutellariae radix were determined by CE with electrochemical detection, the linearity ranges were 0.0010–1.0, 0.0005–1.0 and 0.0005–1.0 mmol l^{-1}, the limits of detection were 0.548, 0.224 and 0.274 μmol l^{-1}, the recoveries were 95.24, 99.08 and 97.52%, and the R.S.D.s were 2.79, 0.94 and 4.76%, respectively [83]. The method was less sensitive than the HPLC with electrochemical detection [59], but it was more sensitive than the HPLC with UV detection [47]. For determination of baicalin in prescriptions containing *S. baicalensis* Georgi by high-performance capillary electrophoresis with UV detection, the linearity range was 10–640 mg l^{-1}. The recoveries were 100.3–100.6% with the R.S.D. of <5% [86]. For determination of baicalin and chlorogenic acid in Yinhuang granules by capillary electrophoresis with UV detection, the calibration graphs were linear from 160–960 and 80–960 mg l^{-1}, respectively. The recoveries were 99.8–102.1% with the R.S.D. of 0.87–3.09% [87]. When six flavonoids in Scutellariae radix were simultaneously determined by MEKC with UV detection, the calibration graphs were linear for 0.007–0.135 mg ml^{-1} of baicalein, wogonin and oroxylin A 7-*O*-glucuronide, 0.013–0.234 mg ml^{-1} of wogonin 7-*O*-glucuronide, 0.025–0.450 mg ml^{-1} of baicalin and 0.005–0.09 mg ml^{-1} of oroxylin A. The recoveries were 98.1–102.6% and the R.S.D.s were 0.9–2.4% [92].

For determination of wogonin in rat plasma by liquid chromatography-tandem mass spectrometry, the linearity range was 0.25–20 ng ml^{-1}. The limit of detection was 0.25 ng ml^{-1} and the within- and between-day R.S.D.s were 2.2–19.1 and 5.9–7.3%, respectively [98]. The HPLC–MS [98] was the most sensitive, followed by the HPLC with electrochemical detection [59], and the CE with electrochemical detection [83]. The HPLC–UV [47] was the least sensitive.

6. Evaluation of the analytical results

Analytical methodology is an essential component not only in the developmental phase of a drug substance but also in the continuing evaluation of clinical efficacy. The HPLC

methods have been applied to pharmacokinetic studies. The pharmacokinetics of wogonin in rats was studied by HPLC after a dose of 5 mg kg^{-1} by intravenous administration [101]. A biphasic phenomenon with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration time profile. In another report, the metabolism of baicalin in human was studied by HPLC after oral administration of Sho-Saiko-To [102]. Baicalin is one of the major components of Sho-Saiko-To. Baicalein 6-*O*-sulfate was identified as the metabolite of baicalin. Baicalein 6-*O*-sulfate was detected after 1 h, reached a maximum level at 5 h and then decreased to the level less than the quantitative limit (5 ng ml^{-1}) after 36 h, and the plasma level of baicalein 6-*O*-sulfate showed a small peak at 24 h. Baicalin was detected after 1 h, reached a maximum level at 5 h and then decreased to the level less than the quantitative limit (5 ng ml^{-1}) after 36 h, and the plasma level of baicalin showed two peaks at 12 h and 24 h.

The metabolic pharmacokinetics of baicalin and baicalein in rats were compared [103]. After intravenous administration of baicalein, 75.7% of the dose was circulated as its conjugated metabolites. After oral administration of baicalein, absorption of baicalein itself was negligible, whereas the glucuronides/sulfates of baicalein were predominant in the plasma. When compared with intravenous bolus administration with dose correction, the absolute absorption was 40%. When baicalin was administered orally, glucuronides and sulfates of baicalein were exclusively circulated in the plasma. The relative absorption for baicalin was 65% when compared with baicalein. Profound differences of serum profile and pharmacokinetics were observed between oral baicalein and baicalin. Baicalin demonstrated significantly a time lag to achieve the peak concentration t_{max}, and the lower peak serum concentration (C_{max}) of baicalein conjugated metabolites compared to baicalein, indicating baicalin was absorbed more slowly and to poorly than baicalein. In addition, the urinary pharmacokinetics of baicalein, wogonin and their glycosides in humans were studied after oral administration of Scutellariae radix [104]. Ten healthy male volunteers received a dose of 5.2 g commercial powder (comparable to 9 g crude drug), respectively. The mean cumulated renal excretion of baicalein glucuronides and sulfates were 43.1 ± 4.5 μmol (2.9% of dose) and 64.8 ± 6.3 μmol (4.3% of dose), respectively, whereas wogonin glucuronides and sulfates were 21.6 ± 2.0 μmol (5.9% of dose) and 20.7 ± 1.7 μmol (5.7% of dose), respectively. The renal excretion of conjugated metabolites of wogonin (11.6% of dose) was higher than that of baicalein (7.2% of dose). The baicalein sulfates was predominant than the corresponding glucuronides, whereas wogonin sulfates was comparable to the corresponding glucuronides.

The metabolism of active constituents in Huangqin-Tang, a prescription in traditional Chinese medicine, was studied by human intestinal flora [105]. Huangqin-Tang and all individual herbs in the decoctions were incubated with a human fecal suspension separately. The active constituents of Huangqin-Tang, including baicalin, wogonoside, oroxylin-A glucuronide, paconiflorin, liquiritin, isoliquirtin and glycyrrhizic acid, were converted to their metabolites baicalein, wogonin, oroxylin-A, paeonimetabolin-I, liquiritigenin, isoliquiritigenin and glycyrrhetinic acid by human intestinal flora. The contents of the metabolites in Huangqin-Tang and in each single herb decoction increased significantly after incubation with intestinal flora. Comparing with single herb decoctions, the transformation of baicalin, wogonoside, oroxylin-A glucuronide, liquiritin and isoliquirtin in the compound prescription was promoted, however, that of paconiflorin and glycyrrhizic acid was inhibited. All the results suggested that the glycosides of many medicinal herbs could be converted to aglycones by human intestinal flora, and the metabolism of most glycosides was improved in the compound prescription. In another report, the pharmacokinetics of multi-constituents in Huangqin-Tang decoction in rats were studied [106]. At different intervals after oral administration of the Huangqin-Tang decoction or a single herb decoction at a dose of 10 g kg^{-1}, the concentrations of the constituents and their metabolites, baicalin, wogonoside, oroxylin-A glucuronide, baicalein, wogonin, oroxylin-A, paeoniflorin (PF), paeonimetabolin-I, liquiritin, liquiritigenin, glycyrrhizic acid and glycyrrhetinic acid, were detected in the rat plasma by HPLC. There were obvious differences in the pharmacoldnetic parameters of most constituents (especially constituent wogonoside) between the compound prescription and single herb decoction. The constituents in the compound prescription had delayed absorption and elimination, a longer residence time in the body, and higher C_{max} and AUC (0-lim), than those in the single herb decoction. Therefore, they were more efficient and durable, making them promising to exerting pharmacological effects in vivo.

The degradation of thirteen flavonoid aglycones-wogonin, diosmetin, hesperetin, baicalein, morin, genistein, daidzein, quercetin, naringenin, luteolin, kaempferol, apigenin and neophellamuretin were investigated in rabbit, rat and human fecal flora suspensions as well as in artificial intestinal juice, using HPLC [107]. The result indicated that all flavonoid aglycones except baicalein, diosmetin and quercetin were stable in artificial intestinal juice, whereas all were degraded in rabbit, rat and human feces suspension. Wogonin and diosmetin were among the less degraded ones for all three feces tested. The presence of a methoxy group on the A or B ring of the flavonoid seems to protect the structure from bacterial degradation. In addition, the bioavailability of baicalin–phospholipid complex was also studied by HPLC [108]. A complex of baicalin with soy phospholipid was prepared to improve the bioabsorption of baicalin. The concentration of baicalin in rat plasma reached a peak of 0.42 μg ml^{-1} at 5.3 h after oral administration, 600 mg kg^{-1}. However, after intake of its phospholipid complex, a peak of 0.90 μg ml^{-1} occurred at a later time, 6.1 h. The elimination of the complex tended to be slower than that of the free drug. There was a significant difference in the mean area under the

concentration–time curves (AUC) between the free drug and the complex ($P < 0.01$).

The HPLC method has been used for quality control of medical products based on *S. baicalensis* Georgi by the determination of baicalin content in Pharmacopoeia of the People's Republic of China [2]. In addition, the fingerprinting technique on HPLC–UV has been used for quality control of raw medical material of *S. baicalensis* Georgi [109,110].

In order to explore more effective herbal products based on *S. baicalensis* Georgi, more widely pharmacological studies should be carried out to determine new pharmacodynamic effects, such as anti-SARS coronavirus effect of baicalin [18]. Secondly, more attention should be paid to minor components in *S. baicalensis* Georgi because special pharmacodynamic effects may be found from minor components. Finally, bioactive components at highly purity should be used instead of crude extracts in medicinal preparations. Therefore, separation and purification techniques will play an important role in these studies.

7. Conclusions

S. baicalensis Georgi is widely explored for the treatment of inflammation, cancers, bacterial infections, and a variety of other diseases. A wide range of separation, detection, and identification methods have been developed and employed for quantitative and qualitative determination of its main active components, baicalin, baicalein, wogonin and oroxylin A. Different sample preparation techniques such as supercritical fluid extraction, pressurized hot water extraction and solid-phase extraction have been also utilized prior to analysis.

High-performance liquid chromatography with ultraviolet detection is the most frequently employed for the determination of these active components in various matrices. The development of HPLC–mass spectrometry coupling is a major advance, offering greater sensitivity than ultraviolet detection and greater selectivity through selected ion monitoring or by using tandem mass spectrometry and selected reaction monitoring mode. These techniques are efficient for identification of these active components and their metabolites in complex matrices because of their excellent specificity, sensitivity and speed. Capillary electrophoresis, micellar eletrokinetic capillary eletrochromatography, and thin layer chromatography are also often applied to the separation of these active components. High-speed counter-current chromatography has been used for the preparative separation and purification of baicalin from Scutellariae radix.

In the future, the automated high-throughput sample preparation in conjunction with parallel column HPLC with tandem mass spectrometry detection will play a key role in quantitative pharmaceutical and biological analyses of these active components. Combined use of HPLC–MS and HPLC–NMR will be the most efficient approach for identification of these active components and their metabolites in complex matrices. High-speed counter-current chromatography will be more widely used for the preparative separation and purification of *S. baicalensis* active components on a large scale.

8. Nomenclature

CE	capillary electrophoresis
GC	gas chromatography
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HSCCC	high-speed counter-current chromatography
MEKC	micellar electrokinetic capillary chromatography
MS	mass spectrometry
NMR	nuclear magnetic resonance
PHWE	pressurized hot water extraction
PLE	pressurized liquid extraction
R.S.D.	relative standard deviation
SARS	severe acute respiratory syndrome
SDS	sodium dodecyl sulphate
SFE	supercritical fluid extraction
SPE	solid-phase extraction
SRM	selected reaction monitoring
TLC	thin layer chromatography
UV	ultraviolet

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