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Identification and quantification of eight flavones in root and shoot tissues of the medicinal plant Huang-qin (*Scutellaria baicalensis* Georgi) using high-performance liquid chromatography with diode array and mass spectrometric detection

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Abstract

A method of analysis of eight flavones using high-performance liquid chromatography (HPLC)-diode array detection (DAD)-mass spectrometry (MS) in root and aerial tissues of the medicinal plant *Scutellaria baicalensis* was developed. The identity of the analytes was confirmed using retention time, UV–vis and mass spectral comparisons to commercial standards. Both UV–vis and mass spectral patterns were characterized for glycosylated flavones. Two additional flavone glycosides were tentatively identified as chrysin-7-glucuronide and wogonoside, but not quantified. Greenhouse and in vitro-grown tissues were analyzed with flavone concentrations ranges of 0.14–150 and 0.030–1.7 μg/mg for greenhouse root and shoot tissue, respectively, and 0.0068–6.4 and 0.082–1.5 μg/mg for in vitro-grown roots and shoots, respectively.

Keywords: *Scutellaria baicalensis*; Flavones

1. Introduction

Plants produce a vast array of natural products, including secondary metabolites, some of which exhibit pharmacological activities. Thus, characterization of medicinal plants through biochemical profiling of bioactive and/or marker compounds has become increasingly important. One class of natural products, ubiquitous in vascular plants, is the flavonoids, which are made up of over 8000 compounds comprising 12 subclasses including flavones, flavanones and flavanols [1,2]. The medicinal and pharmacological activities of flavonoids against inflammation, allergies, viruses, cancer and other ailments are well documented [1,3].

Huang-qin (*Scutellaria baicalensis* Georgi) is an important medicinal herb, with potential commercial production, that has been used for treatment of various ailments including fevers, ulcers, cancers, and inflammation [4,5]. Flavonoids are the main active components in Huang-qin with over 40 structures identified [6,7]. Many flavones with bioactivities have been identified in the tissues of *S. baicalensis*. Baicalin, baicalein, wogonin, and wogonoside are four flavones with antioxidant activity and are considered major components [6,8]. Other flavones found in Huang-qin with bioactivities include apigenin, chrysin, and scutellarein [3,9,10].

Previous separation methods of flavonoids in *S. baicalensis* have included capillary electrophoresis [11], thin-layer chromatography [10,12], gas chromatography [12], and column chromatography [10]; however, reverse-phase high-performance liquid chromatography (HPLC) has been the most widely used method of flavonoid analysis because of

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the polarity and low volatility of flavonoids [13]. High-performance liquid chromatography is an especially powerful tool when combined with diode-array detection (DAD), and mass spectrometry (MS). These two detectors can provide comprehensive qualitative, using spectral characteristics, and quantitative information. Increasingly, mass spectrometry, using API interfaces, is used for flavonoid analysis because of its sensitivity and selectivity: the ability to characterize and quantify the wide range of flavonoid classes and polarities [13,14].

By combining HPLC, DAD, and MS for medicinal plant analysis, the identification and quantification of bioactive and marker compounds in complex matrices can be realized even with structurally similar natural products [15]. The purpose of this study was to develop a quantitative and qualitative analytical method of eight bioactive flavones in root and shoot tissues of *S. baicalensis* using HPLC, DAD, and ESI positive MS. This method was used for subsequent quantitative analysis of two different *S. baicalensis* plant types, including greenhouse and in vitro-grown plants, to establish potential differences of flavone concentrations between the two plant types.

2. Materials and methods

2.1. Chemicals

Optima grade methanol (MeOH) was purchased from Fisher (Nepean, ON, Canada). Acetonitrile (ACN) and formic acid, 88% in water, were obtained from Caledon Laboratories (Georgetown, ON, Canada). Ultrapure water at 18.3 MΩ resistance, used for extractions and HPLC mobile phase, was prepared using a Nanopure (New Haven, CT, USA) filtration system.

Flavone standards, as seen in Fig. 1, included wogonin (99.5%), baicalin (99%), baicalein (98%), and scutellarein (99%), which were purchased from Chromadex (Laguna Hills, CA, USA); apigenin-7-glucuronide (api-glcA) from Apin Chemicals (Oxon, UK); scutellarin from Herbstandard (Chesterfield, MO, USA); apigenin (95%) from Sigma (Oakville, ON, Canada); chrysin and the surrogate standard 6-hydroxyflavone were obtained from Indofine (Hillsborough, NJ, USA). All standards were dissolved in MeOH and stored in dark at 4 °C.

2.2. Sample preparation

Greenhouse-grown plants of *S. baicalensis* were obtained from Richters Herb's (Goodwood, ON, Canada). Two cell lines, #106 and #111 of in vitro-grown *S. baicalensis* tissue were obtained from Dr. Praveen Saxena of the University of Guelph. Both the shoot and root tissue were separated and freeze-dried using a Labconco Freeze Dry System, from Caltec Scientific Ltd. (Toronto, ON, Canada), at 10×10^{-3} mbar and −40 °C. Freeze-dried tissue was homogenized in a blender and stored at −80 °C until analysis. Root, 0.2 g, and shoot, 0.5 g, tissues were extracted, separately, in 100 ml of 70:29:1 (v/v/v) MeOH:water:formic acid in an ultra-sonic bath for 120 min at 65 °C. Extracted samples were filtered through 0.45 μm PTFE filters using Whatman (Kent, UK) mini uniprep autosampler vials.

2.3. HPLC-DAD-MS

The HPLC system was an Agilent Technologies (Mississauga, ON, Canada) Series 1100 consisting of a binary pump, continuous vacuum degasser, thermostated autosampler, and column compartment coupled to a variable wavelength diode-array detector and an SL quadrupole MS with an ESI interface. A Waters (Missisauga, ON, Canada) SymmetryShield RP-18 (100 mm × 2.1 mm × 3.5 μm) column was combined with a guard column of the same stationary phase (10 mm × 2.1 mm × 3.5 μm) and a 0.5 μm pre-column filter from Supelco (Oakville, ON, Canada), all heated to 30 °C in the column compartment was used for all analyses. The sample compartment was held at 4 °C and the injection volume of standards and samples was 5 μl. Chromatographic separation of flavonoids used a gradient, listed in Table 1, of solvent A: 0.1% (v) formic acid in water and solvent B: ACN with 0.1% (v) formic acid at a flow rate of 0.4 ml/min. All mobile phase solvents were filtered with a Whatman 0.45 μm nylon filter prior to use.

The DAD detected analytes at 278 nm with 580 nm reference wavelength, both at 4 nm bandwidth, with full spectral scanning from 200 to 425 nm and 0.5 nm resolution using a semi-micro flow cell. Fig. 2 shows the DAD chromatogram of a mixed flavone standard including the surrogate standard, 6-hydroxyflavone.

The MS used two signals of detection for analysis. The first signal was in positive scan mode with 100–500 amu range at 175 V fragmentor voltage. The protonated molecular ions, $[M+H]^+$, of the flavone analytes were used for selected ion monitoring (SIM) mode for the second MS signal. For the SIM signal the dwell time was 45 ms at 70 V fragmentor voltage. For both MS signals, the capillary voltage was 4 kV, and the gain was 4.0. The cycle time was 0.82 s/cycle, split 50% for each of the two MS signals. Nitrogen, from BOC

Table 1
HPLC gradient program for flavone analysis

Time (min)[a]	Solvent A (%)[b]	Solvent B (%)[c]
0.00	70	30
2.00	70	30
6.00	40	60
11.00	15	85
11.25	1	99
12.25	1	99
12.50	70	30
16.00	70	30

[a] A post time of 1 min was used.
[b] 0.1% (v) formic acid in water.
[c] Acetonitrile with 0.1% (v) formic acid.

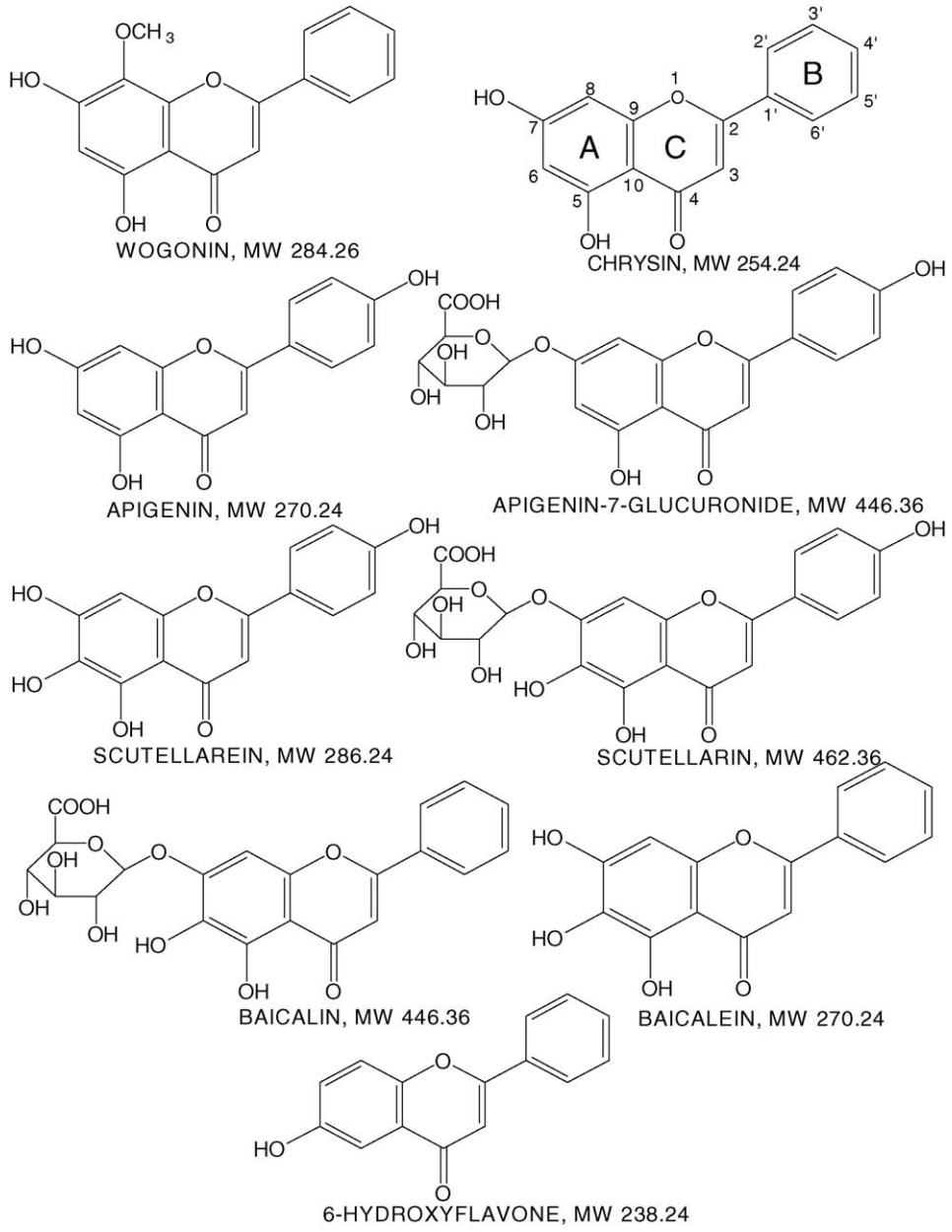

Fig. 1. The structures and molecular weights of the flavone analytes and 6-hydroxyflavone.

Gases (Mississauga, ON, Canada), was used as a drying gas at a flow rate of 11.5 l/min, temperature 350 °C and a nebulizing pressure of 35 psi. Control for the HPLC, DAD, and MS was by Agilent's ChemStation LC/MS R.A08.04 software.

2.4. Method validation

Percent recovery experiments of the surrogate standard, 6-hydroxyflavone, were completed. Three concentration levels, 3.7, 15, and 37 μg/ml, were spiked in triplicate in root and shoot tissues. Percent recovery of the flavone analytes was determined by spiking both matrices, in triplicate, with the available standards at varying concentrations (see Table 2). The percent recoveries for the analytes were calculated by subtracting the spiked concentrations from non-spiked matrix samples using external standard linear regression. All recovery experiments used aliquots of plant sample from the same homogenized plant. All analytes except baicalin were spiked in root samples and all but scutellarin, apigenin, and api-glcA were spiked in shoot samples.

An additional experiment determined the extraction efficiency by a sequential extraction of each matrix three times. Extraction repeatability, precision, was established by extracting three samples of both matrices once and determining the percent R.S.D. of the analyte concentrations for the three extractions.

Table 2
Percent recovery determinations using shoot and root matrices

Matrix	Flavone	Weight of standard added to matrix (μg, $n = 3$)	Average recovery (%)	R.S.D. (%)
Shoot[a]	Scutellarein	320	96	6.4
	Baicalein	200	110	3.4
	Apigenin	300	86	2.5
	Wogonin	40	72	0.5
	Chrysin	100	79	0.5
	6-Hydroxyflavone[c]	350	102	1.8
		1500	100	0.4
		4000	91	1.7
Root[b]	Scutellarin	300	78	1.8
	Api-glcA	50	125	4.5
	Scutellarein	150	107	8.5
	Baicalein	800	116	1.5
	Wogonin	120	94	1.6
	Chrysin	50	77	1.9
	6-Hydroxyflavone[c]	340	110	1.6
		1500	97	0.6
		4000	93	0.3

[a] Scutellarin, api-glcA, and baicalin not quantified in shoot matrix.
[b] Baicalin and apigenin not quantified in root matrix.
[c] 6-Hydroxyflavone was tested at three different concentrations.

2.5. Quantitative analysis

External standard least squares linear regression, with at least five concentration points, was used for quantification. Calibration curves for all eight analytes were made at different concentration ranges (Table 3) and tested using three detection signals: MS-extracted ion chromatogram (EIC), MS-SIM and UV–vis response. The EIC signal was obtained at a specific m/z value corresponding to $[M + H]^+$ of the analyte. Linearity was assessed using response factors (RF) and the regression coefficient (r^2) separately. Response factors were calculated using the following equation:

$$RF = \frac{DR}{C} \quad (1)$$

where DR is the detector response, in area counts, and C is the concentration of the analyte injected. The detection signal that produced the best linear calibration curve for individual analytes was subsequently chosen for quantitative analysis.

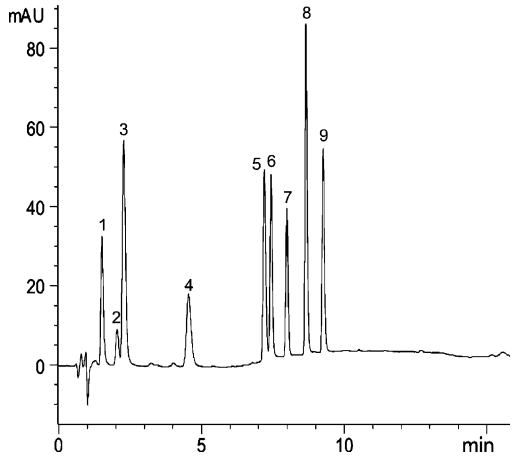

Fig. 2. HPLC-DAD chromatogram of the flavone standards at 278 nm (reference 580 nm): (1) scutellarin, $t_r = 1.51$; (2) apigenin-7-*O*-glucuronide, $t_r = 2.04$; (3) baicalin, $t_r = 2.27$; (4) scutellarein, $t_r = 4.54$; (5) baicalein, $t_r = 7.20$; (6) 6-hydroxyflavone, $t_r = 7.44$; (7) apigenin, $t_r = 7.99$; (8) wogonin, $t_r = 8.66$; (9) chrysin, $t_r = 9.26$ min.

3. Results and discussion

3.1. Optimization of flavone standard separation

Fig. 2 shows the gradient separation of the flavone and surrogate standards at 278 nm. With respect to elution order, the three flavones with a polar 7-*O*-glucuronic acid moiety, scutellarin, apigenin-7-*O*-glucuronide, and baicalin, were first to elute followed by the less polar aglycone flavones, as expected. Conversely, the magnitude of separation and order of some of the aglycones was unexpected. Scutellarein was the only flavone to elute between 3 and 7 min; however, it only contained one more hydroxyl group than baicalein and apigenin. In addition, the surrogate standard 6-hydroxyflavone had only one hydroxyl moiety, yet it eluted prior to the flavones that contained two hydroxyl groups, chrysin and wogonin, or three hydroxyl groups, apigenin. These results may be attributed to the absence of an A-ring, C-5 hydroxyl group in 6-hydroxyflavone, which is present in apigenin, wogonin, and chrysin, and provides an intramolecular hydrogen bond between the C-4 carbonyl group and the C-5 hydroxyl group (see Fig. 1). The presence of this hydrogen bond seems to reduce the polarity of the flavones as the chemical interaction of the polar hydroxyl group, at C-5, and

Table 3
Linear calibration information and data for the flavone analytes

	Quantification signal	Calibration range (μg/ml)	Response factor R.S.D. (%)	r^2
Scutellarin	MS-EIC[a,c]	1.1–65	5.9	0.997
Api-glcA	MS-SIM[b]	0.20–20	11.8	0.998
Baicalin	DAD	0.50–102	6.8	0.998
Scutellarein	MS-SIM[b]	1.0–20	11.8	0.978
Baicalein	DAD	2.3–38	9.7	0.999
6-Hydroxyflavone	DAD	5.9–73	7.2	0.995
Apigenin	DAD	0.30–31	6.0	0.999
Wogonin	DAD	0.30–30	2.1	0.999
Chrysin	DAD	0.30–25	3.9	0.999

[a] Mass spectrometer-extracted ion chromatogram.
[b] Mass spectrometer-selected ion monitoring.
[c] Quantified using *m*/*z* 463 as extracted ion.

the polar mobile phase is reduced. Therefore, the substitution pattern of the hydroxyl groups affects the elution profile of the flavones.

Initial development and optimization of the gradient separation was to achieve sufficient resolution among flavones for accurate and precise quantification while maintaining a reasonably fast gradient time. Because *S. baicalensis* has potential in commercial nutraceutical production, this goal was based on the succeeding use of this method to screen multiple *S. baicalensis* plants grown under various growth conditions in future experiments. The separation of the analytes was complete in less than 10 min with additional time required to clean and re-equilibrate the column. This separation time is an improvement over previous flavonoid separations, which have varied between 20 and 50 min for gradient separations of less than five flavonoids [16–19].

3.2. Validation

An efficient way of establishing the accuracy of extraction is to determine the percent recoveries of spiked analytes [20]. Table 2 shows these results in both matrices of *S. baicalensis*. In shoot tissue, the percent recoveries ranged between 72 and 110%; in root tissue between 77 and 125% for the given analytes. The recovery R.S.D. for both matrices did not exceed 9%.

One difficulty in determining percent recovery for endogenous compounds in medicinal plants is that no blank matrix is available. 6-Hydroxyflavone was chosen as a surrogate standard because of its structural similarity to the other analytes; thus, the standard recoveries served as an indication of extraction efficiency for the other flavone analytes that were not tested. Percent recoveries of 6-hydroxyflavone ranged between 91 and 102% for shoot and between 93 and 110% for root tissue with R.S.D. below 2% (Table 2).

Extraction efficiency was determined for both matrices using three successive extractions. Carryover was observed in the second extraction with baicalin (1.1%), scutellarin (0.6%), and chrysin (1.2%) in shoot tissue, while in root tissue only baicalin was present (0.3%). These results are reported as a percentage of the concentration determined in the first extraction. No analytes were detected in the third successive extraction.

In addition to accuracy, extraction repeatability was determined by extracting three samples once with subsequent analysis. Table 4 shows the extraction repeatability percent R.S.D. for the three sample extractions with an average percent R.S.D. of 3.6% for shoot and 4.0% for root tissue.

3.3. Calibration

Table 3 shows the least-squares linear calibration data for the flavone analytes. Because both the DAD and MS were used, three detector signals were available for flavone quantification including: DAD response, MS-SIM, and MS-EIC. Two parameters were used to assess linearity: regression coefficient, r^2, and response factor. In most cases, r^2 is an acceptable means of evaluating linearity for regression; however, problems can arise with r^2 values. These problems tend to be magnified at low and high calibration concentrations where deviations from linearity and confidence intervals increase and are especially evident when using large calibration ranges over several orders of magnitude [20,21]. A more accurate and sensitive method of quantifying linearity is to use response factors (Eq. 1), which are not susceptible to the

Table 4
The extraction repeatability for flavones in root and shoot matrices of *S. baicalensis* with corresponding concentrations

Matrix	Analyte	Average concentration (μg/ml, $n = 3$)	R.S.D. (%)
Shoot	Scutellarein	5.0	4.0
	Baicalein	2.5	3.4
	Apigenin	2.0	3.5
	Wogonin	0.15	2.3
	Chrysin	8.5	4.6
Root	Scutellarin	9.0	4.8
	Api-glcA	0.60	8.7
	Baicalin	300	3.3
	Scutellarein	2.4	2.3
	Baicalein	9.4	3.6
	Wogonin	1.4	0.4
	Chrysin	0.28	4.9

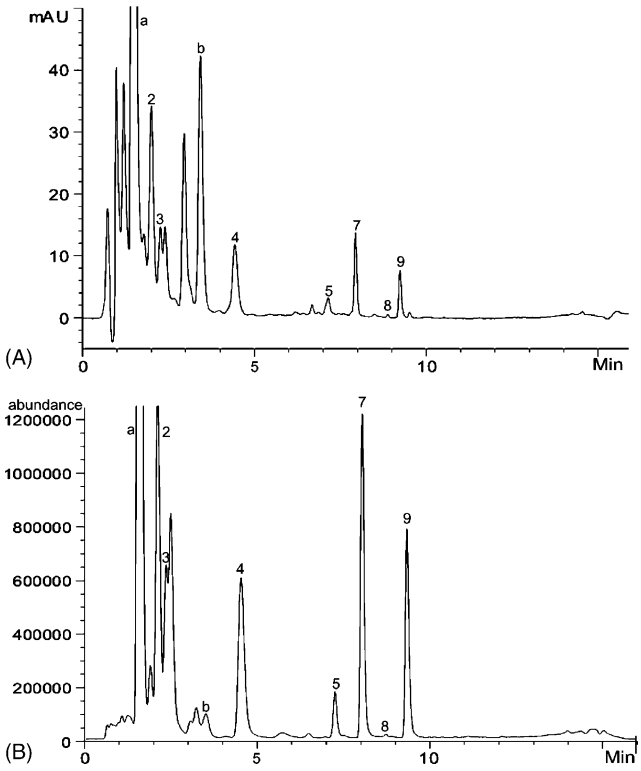

Fig. 3. DAD (A) and MS-SIM (B) chromatograms of a greenhouse-grown shoot extract of *S. baicalensis*. Peak numbers correspond to flavone analytes presented in Fig. 2. Peaks (a) and (b) are the scutellarin co-elution peak and an unknown that was further investigated for structural identification, respectively.

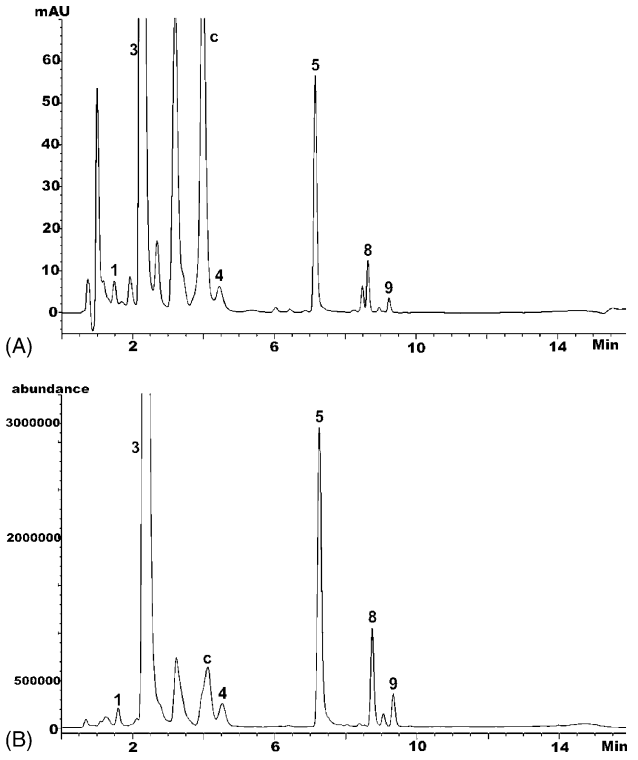

Fig. 4. DAD (A) and MS-SIM (B) chromatograms of a greenhouse-grown root extract of *S. baicalensis*. Peak numbers correspond to flavone analytes presented in Fig. 2. Peak (c) is an unknown that was further investigated for structural identification.

potential problems of r^2 [20]. Ideally, the response factor values should be equal throughout the calibration range; thus, lower % R.S.D.s of response factors equals better linearity.

3.4. LC-DAD-MS of S. baicalensis

Eight flavone analytes were quantified in greenhouse and in vitro-grown tissues of *S. baicalensis* using both DAD and MS detection. Figs. 3 and 4 show DAD and MS-SIM chromatograms of greenhouse-grown shoot and root tissue, respectively. The complexity of both matrices was markedly different between the polar and non-polar areas of the separation for each matrix (gradient elution). The shoot tissue extract chromatogram (Fig. 3) showed a complex polar fraction resulting in the increased collection of peaks within the first 3 min.

To identify the flavones in each matrix, retention times, UV–vis and mass spectral characteristics were compared to commercial standards. This resulted in the recognition of specific spectral patterns between the glycosylated flavones and their respective aglycones: scutellarin, scutellarein; apigenin-7-*O*-glucuronide, apigenin; and baicalin, baicalein. For the preceding flavone pairs, glycone versus aglycone, the λ maxima values were the same as the glucuronic acid moiety provided little contribution to the UV absorbance pattern due to lack of chromophores. Scutellarin and scutellarein had λ maxima of 283 and 334 nm; apigenin-7-*O*-glucuronide and apigenin had λ maxima of 267 and 335 nm; and baicalin and baicalein had λ maxima of 267 and 335 nm (spectra not shown). In addition, a mass spectral pattern was determined between the same three flavone pairs. Mass spectra of each flavone pair differed by only one ion peak: the $[M+H]^+$ of the glycosylated flavone. Each of the three glycone analytes' spectra contained the $[M+H]^+$ and an intense aglycone ion peak, $[A+H]^+$, corresponding to the neutral loss of a glucuronic acid moiety, 176 amu, from parent (spectra not shown). This neutral loss fragmentation pattern was attributed to in-source CID due to the high fragmentor voltage. Recent investigations by Hvattum [22] and Cuyckens et al. [23] successfully utilized this technique of in-source CID for structural elucidation of various conjugated flavonoids, including glycosylated flavonoids.

Wogonin and chrysin, both aglycones, did not have a corresponding commercial standard with a glucuronic acid moiety attached in the C-7 position. Wogonin, showed one UV–vis λ maximum at 275 nm (spectrum not shown). The mass spectrum showed a $[M+H]^+$ at 285 amu with one fragment ion at 270 amu corresponding to the neutral loss of $\cdot CH_3$ from the C-8 position on the A-ring. In previous studies, methoxylated flavonoids have exhibited this $\cdot CH_3$ neutral loss in both positive [24] and negative [25] modes. When using single quadrupole MS, the occurrence of

Table 5
Flavone concentrations, in μg analyte/mg sample (dry weight), quantified in greenhouse and in vitro-grown plant types of *S. baicalensis*

Tissue type	Flavone	Greenhouse tissue concentration (μg/mg)	In vitro cell line #106 concentration (μg/mg)	In vitro cell line #111 concentration (μg/mg)
Shoot	Scutellarein	1.0	0.35	0.13
	Baicalein	0.50	0.22	0.33
	Apigenin	0.40	0.089	0.082
	Wogonin	0.030	0.16	0.14
	Chrysin	1.7	1.4	1.5
Root	Scutellarin	4.5	ND[a]	0.16
	Api-glcA	0.30	0.0068	ND[a]
	Baicalin	150	1.4	6.4
	Scutellarein	1.2	0.047	0.021
	Baicalein	4.7	0.28	1.2
	Wogonin	0.70	0.20	0.61
	Chrysin	0.14	0.28	0.34

[a] Not detected.

$\cdot CH_3$ fragmentation is dependent on the magnitude of the fragmentor voltage, which determines the extent of in-source CID. The other aglycone, chrysin, showed two λ maxima at 268 and 313 nm in the UV–vis spectrum and a $[M+H]^+$ at *m*/*z* 255 with no fragment ions in the mass spectrum.

3.5. Flavone concentrations in S. baicalensis

The flavone analytes quantified in both greenhouse and in vitro-grown tissues of *S. baicalensis* are listed in Table 5. In root tissue, the major component was baicalin, for both plant types, with a concentration of 150 μg/mg in greenhouse-grown tissue. All other flavone concentrations were markedly lower. A previous investigation by Lin et al. [18] used ultrasonic shaking and a MeOH–water (70:30) solvent to extract baicalin, baicalein, and wogonin from roots producing concentrations of 113.5, 5.7, and 2.3 μg/mg (dry weight), respectively [18]. Zhang et al. [16] also analyzed flavones in *S. baicalensis* roots with reported flavone concentrations (approximate) of 144 μg/mg baicalin, 29.9 μg/mg baicalein, and 9.7 μg/mg wogonin (dry weight), using a reflux extraction of 50% ethanol. The differences in flavone concentration among the two previous reports and the present method are not unexpected as growth conditions varied for the preceding two studies and the present study. However, the trend of flavone concentrations is consistent among the three studies showing baicalin ≫ baicalein > wogonin [18,16].

In shoot tissue, the flavone concentrations varied with no obvious trends between greenhouse and in vitro tissue (Table 5). In a previous study by Nishikawa et al. [4], baicalin, baicalein and wogonin were tested, but not detected in the leaves and stems of *S. baicalensis* plants grown in vitro. However, the method of flavonoid analysis used by Nishikawa et al. [4] may not have been sensitive enough to detect low concentrations of baicalin, baicalein and wogonin in the tissue as UV–vis detection was used. This observation remains unknown as no method validation information was presented. For both plant types tested, four of five and six of seven flavone analytes, in shoot and root tissue respectively, had higher concentrations in greenhouse versus in vitro-grown plant types. This was consistent with a study by Stojakowska and Malarz [26] and Kuzovkina et al. [27], which found flavonoid concentrations to be much lower in in vitro-grown tissue to that of greenhouse plants of *S. baicalensis*.

3.6. Identification of unknown peaks in S. baicalensis

In both matrices of *S. baicalensis*, there were unknown peaks that were investigated for structural identification. In the shoot chromatograms (Fig. 3), two unknown peaks (a) and (b), were analyzed. Peak (a) was determined to be a co-elution of an intense unknown compound and scutellarin. From this peak, scutellarin could not be fully distinguished as the unknown was substantially more intense than scutellarin, and the peak's mass spectrum (not shown) contained a collection of fragment ions from both compounds. To ameliorate this problem, the peak was isolated by fractionation and subjected to isocratic separation. The fraction was subsequently separated and scutellarin was identified.

The other intense unknown peak investigated in the shoot chromatogram was (b) in Fig. 3. The UV–vis spectrum for (b) showed the same absorbance pattern as chrysin with λ maxima of 268 and 313 nm (spectra not shown). An MS-EIC was obtained for chrysin at *m*/*z* 255, presented in Fig. 5B, and shows two intense peaks. The mass spectrum of (b) showed a base peak $[M+H]^+$ of 431 amu and an intense fragment ion of 255 amu, $[A+H]^+$, corresponding to a neutral loss of 176 amu. Based on the similarity of mass spectral trends to the other glycosylated flavone analytes, previously described, the 176 amu neutral loss represents a glucuronic acid moiety. Therefore, the two chromatographic peaks, corresponded to a polar glycosylated conjugated flavone (b), and the non-polar aglycone, chrysin (peak 9). For comparison purposes, the MS-EIC's were obtained for the other aglycone analytes and produced the same chromatographic and mass spectral pattern. This led to the tentative identification of (b) as chrysin-7-*O*-glucuronide (MW 430.3).

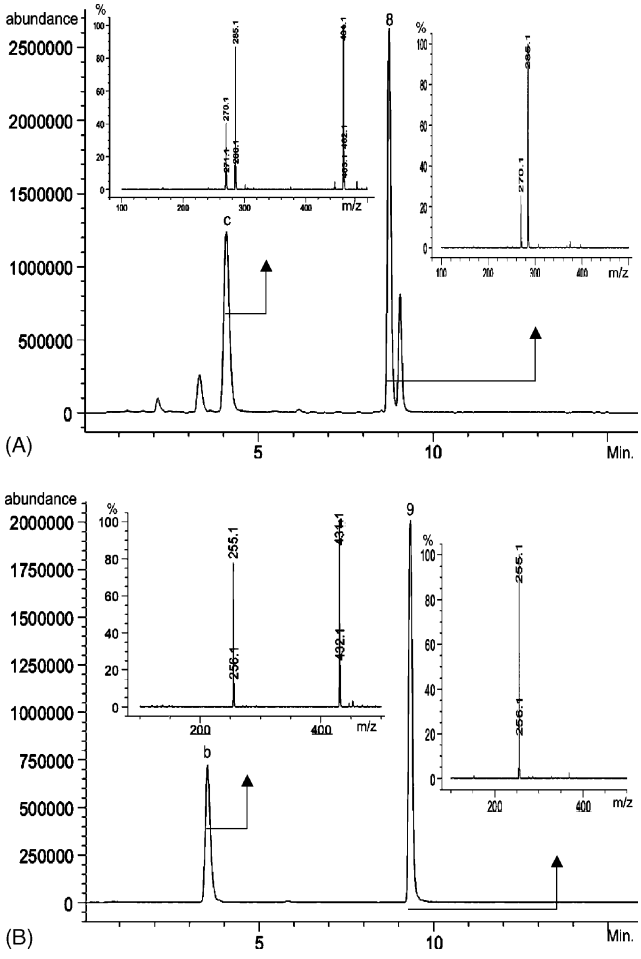

Fig. 5. MS-EIC chromatograms at: (A) *m*/*z* 285 representing wogonin's $[M+H]^+$ ion; and (B) at *m*/*z* 255 representing chrysin's $[M+H]^+$ ion. Mass spectra (arrows) are shown for the unknown peaks (b) and (c) tentatively identified as the glycosylated conjugates, chrysin-7-*O*-glucuronide and wogonoside, respectively, and the aglycones chrysin (9) and wogonin (8).

In root tissue, one unknown peak (c) was further investigated for structural identification (Fig. 4). The absorbance pattern in the UV–vis spectrum of (c) was identical to that of wogonin with a λ maximum at 275 nm (spectra not shown). An MS-EIC was obtained for wogonin at *m*/*z* 285, shown in Fig. 5A. This MS-EIC showed the same chromatographic pattern with (c) and wogonin (peak 8) as with the previous unknown (b) and chrysin (peak 9). The mass spectrum of (c) showed a $[M+H]^+$ at 463 amu with an intense fragment at 285 amu, $[A+H]^+$ corresponding to a neutral loss of 176 amu (Fig. 5A). An additional fragment at 270 amu (∼40% base peak) was present indicating the neutral loss of $\cdot CH_3$ (15 amu) from the methoxy on C-8, ring A; the same trend as seen with wogonin's spectrum. Based on these results, peak (c) was tentatively identified as wogonin-7-*O*-glucuronide, also called wogonoside (MW 460.4). Because no commercial standards were available, both chrysin-7-*O*-glucuronide and wogonoside could not be unequivocally identified in the *S. baicalensis* tissues.

4. Conclusion

A method of flavone analysis was developed for root and shoot tissues of the medicinal plant *S. baicalensis* Georgi using HPLC-DAD-MS. All eight flavone analytes were identified by comparison of their retention times, UV–vis and mass spectral characteristics to available commercial standards. Based on validation results, the developed method proved useful for flavone analysis under the specified conditions. Although DAD was useful for quantification and establishment of UV–vis spectral characteristics of the flavone analytes, MS detection exhibited higher sensitivity for low level analyte concentration determination and qualitative establishment of structural characteristics of flavones. Additionally, MS facilitated determination of qualitative fragment patterns of the glycosylated flavones leading to the tentative identification of two unknown flavones, wogonin-7-*O*-glucuronide (wogonoside) and chrysin-7-*O*-glucuronide, in the tissues of *S. baicalensis*.

Two different tissue types of *S. baicalensis*, including greenhouse and in vitro-grown were quantitatively analyzed for flavone concentration. This method showed good sensitivity as low-level differences in flavone concentrations were observed among the plant types. Establishing good sensitivity was important for testing the two in vitro cell lines, 106 and 111, which produced substantially lower concentrations of flavones, as compared to greenhouse-grown, especially in root tissue.

As consumer usage of medicinal plants increases, more comprehensive quantitative and qualitative testing and biochemical profiling of bioactive compounds will become necessary for quality control purposes. This method should support subsequent flavone analysis of the medicinal plant *S. baicalensis* Georgi.

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