Journal of Chromatography A, 1217 (2010) 1875–1881

Contents lists available at ScienceDirect

Journal of Chromatography A

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

On-line continuous flow ultrasonic extraction coupled with high performance liquid chromatographic separation for determination of the flavonoids from root of *Scutellaria baicalensis* Georgi

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ARTICLE INFO

Article history:
Received 30 September 2009
Received in revised form 8 January 2010
Accepted 18 January 2010
Available online 25 January 2010

Keywords:
Dynamic ultrasonic extraction
Continuous sampling
On-line detection

ABSTRACT

An on-line method, based on coupling dynamic ultrasonic extraction (DUE), continuously sampling the suspension of sample and solvent, high performance liquid chromatographic separation with diode array detection, has been developed for the determination of the flavonoids, including baicalin, baicalein and wogonin, from the root of *Scutellaria baicalensis* Georgi. Variables influencing the DUE were evaluated by orthogonal test. The extraction yields of baicalin, baicalein and wogonin in the roots of *S. baicalensis* Georgi obtained from five different cultivated areas are 73.8–131.5 $\mu g\,mg^{-1}$ (RSD $\leq$ 6.24%), 6.8–15.9 $\mu g\,mg^{-1}$ (RSD $\leq$ 5.36%) and 4.4–14.3 $\mu g\,mg^{-1}$ (RSD $\leq$ 5.30%), respectively. The limits of detection for baicalin, baicalein and wogonin are 0.30, 0.37 and 0.41 $\mu g\,mL^{-1}$, respectively. Linearity is from 0.55 to 109 $\mu g\,mL^{-1}$ for baicalin, from 0.51 to 105 $\mu g\,mL^{-1}$ for baicalein and from 0.53 to 102 $\mu g\,mL^{-1}$ for wogonin. Compared with off-line continuous flow-DUE, the proposed method would be more convenient for the determination of the analytes and the rapid optimization of the extraction process. The extraction yields of flavonoids obtained by the proposed method are comparable with those obtained by dynamic microwave assisted extraction, static ultrasonic extraction and reflux extraction. The result indicated that the proposed method is suitable to determine the active components in Chinese herbal medicine.

1. Introduction

The root of *Scutellaria baicalensis* Georgi is widely used as traditional Chinese herbal medicine. The medical and pharmacological activities of the medicinal plant against inflammation, metamorphosis, viruses, cancer and other ailments have been well documented [1–5]. Generally, the flavonoids, including baicalin, baicalein and wogonin (Fig. 1), are the main bioactive components in *S. baicalensis* Georgi [6–8], and the content of baicalin has been observed in the highest abundance [9–12]. So the content of baicalin is often used as the criterion for estimating the quality of the *S. baicalensis* Georgi.

Soxhelt extraction (SE) and maceration extraction (ME) are often effective but time consuming and labor intensive. Moreover, when the methods are applied, large amounts of solvent and sample are usually required. The ultrasonic extraction (UE) and microwave assisted extraction (MAE) have been applied for more than decades [13–15] mainly due to considerable savings in processing time and solvent consumption [16–19]. The UE and MAE are generally static extractions, which does not involve continuous transport of analytes out of the extraction vessel. One way to overcome this obstacle is to make use of a dynamic approach. The closed [20,21] and open systems [22,23] are two available modes of dynamic extraction systems. Compared with the former, the open dynamic operational mode has the advantages such as facilitating the coupling of extraction with other steps of the analytical process and offering the possibility of determining target compounds on-line. Although dynamic microwave assisted extraction (DMAE) is generally fast and efficient, UE provides several interesting advantages over MAE: (a) the ultrasonic procedure is usually less expensive; (b) in some cases, the ultrasonic procedure is safer than the microwave one, because neither high pressure nor high temperature is present during UE. There were a few reports concerning the application dynamic extraction in medicinal plants [20,21]. However, in most of these studies, the extraction and determination were performed separately. In recent years, an obvious trend is towards on-line systems that integrate the sample preparation and separation and detection of analytes. In an on-line system, the whole analysis is performed in a closed system. For the on-line systems, benefits, such as minimization of manual work, low consumption of sample and solvent, were offered [24,25]. Several texts provide in-depth overviews of the mechanism of extrac-

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doi:10.1016/j.chroma.2010.01.050

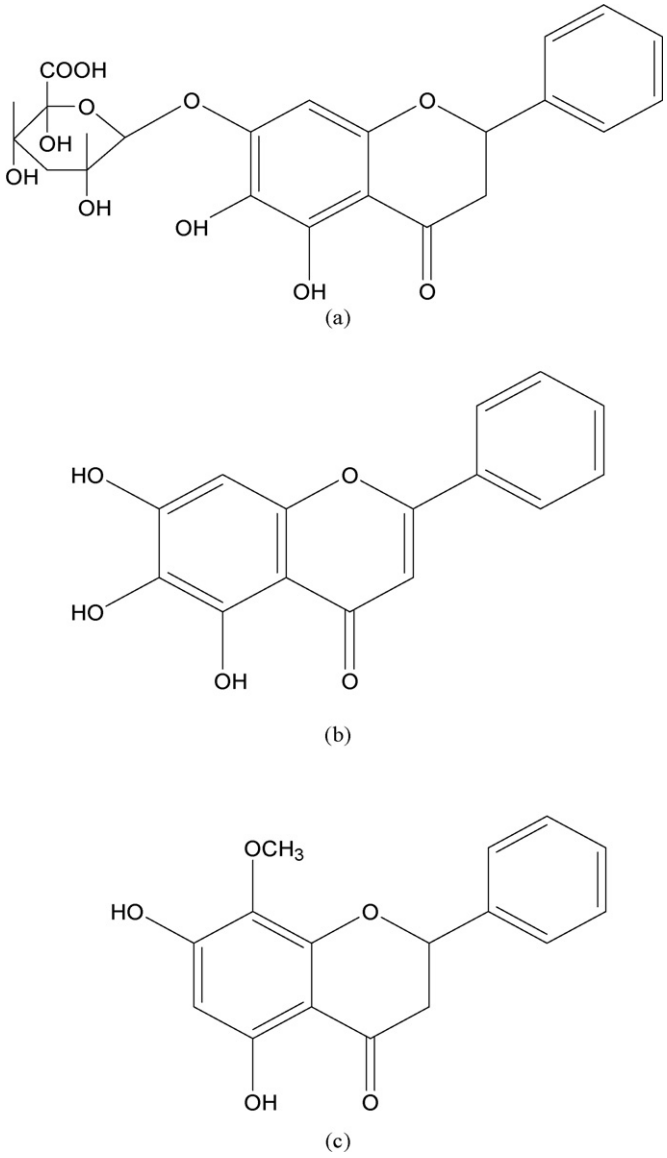

Fig. 1. Structure of baicalin (a), bacalein (b) and wogonin (c).

tions and instrumental aspects for on-line extraction-GC detection [24–31]. In recent years, extraction methods coupled with on-line liquid chromatography (LC) or LC–MS, including supercritical fluid extraction (SFE) [32–36], DMAE [37–39], dynamic ultrasonic extraction (DUE) [40–42], continuous flow liquid membrane extraction [43–46], subcritical water extraction [47], solid-phase extraction (SPE) [48–52] and solid-phase microextraction (SPME) [53,54], have been applied. The basic steps in on-line extraction-liquid chromatographic separation can be described as follows: (1) extract the analytes from the sample, (2) transfer the extract into an intermediate trap (SPE or membrane) or a injector of LC system, and (3) introduce the analytes in the trap or the extract in the injector into a LC system. In addition, other literature about on-line procedure, such as DMAE coupled with atomic absorption [55,56] and UV–Vis spectrophotometric measurement [57], DUE coupled with UV–Vis spectrophotometric measurement [58,59] has also been published. However, most of the methods reported in the literature have the drawback of the complexity of on-line systems. Moreover, in most on-line methods, only the extraction solvent continuously flows but the sample is static and fixed in the extraction vessel. One of the features of the static sampling system is that the fresh extraction solvent is continuously introduced into the extraction vessel. Another feature is that the analytes are transferred out of the extraction vessel instantly. Thus the degradation for the analytes can be avoided, especially for the thermo-liable compounds. However, when the static sampling system is applied, the concentrations of analytes in the extract change and the concentrations of analytes in different portions of extract are different. It is difficult to obtain accurate result by analyzing a portion of extract unless the whole extract is analyzed or all portions of the extract are completely mixed before analysis. On the contrary, when continuous sampling system is applied, in which both the extraction solvent and the sample continuously flow, as long as the suspension is uniform, the analyte concentration in the extract out of the extraction vessel will be constant and it is easy to obtain the accurate result based on the on-line analysis. Additionally, when the static sampling system is applied, the samples must be repeatedly enclosed in and took out of the extraction vessel for analyzing the different samples, and when the continuous sampling system is applied, the analysis of different samples is very convenient [60,61]. Of course when the continuous sampling system is applied, because the suspension is aspirated from a flask and driven through a tubing system to the extraction chamber the reproducibility should be limited.

In this paper, DUE and continuous sampling were on-line coupled with high performance liquid chromatographic separation for the extraction and determination of flavonoids from *S. baicalensis* Georgi. Variables influencing the DUE were evaluated, samples cultivated in different areas were analyzed and the results were compared with those obtained by off-line continuous flow-DUE, DMAE, UE and reflux extraction (RE).

2. Experimental

2.1. Instruments

A continuous sampling DUE system was assembled in our laboratory (Fig. 2). The system mainly consists of an ultrasonic cleaner (KQ2200E Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, China), a micro-infusion pump (WZ-50, Zhejiang medical instrument Co., Ltd., Hangzhou, China) and a peristaltic pump (Michem Technology Co., Ltd., Beijing, China). The frequency and output power of the ultrasonic cleaner are 40 kHz and 150 W, respectively. In the UE experiments, a 100 mL flask was placed in the bath of the ultrasonic cleaner (300 mm × 240 mm × 180 mm). A polytetrafluoroethylene coil (320 cm × 3 mm i.d.) was used as extraction coil. The syringe PTFE filter (0.45 μm, 25 mm i.d.) was placed at end of the extraction coil to finish the on-line filtration. The 1100 series liquid chromatograph (Agilent technologies Inc., USA) equipped with photodiode-array detector (DAD) and quaternary gradient pump was used. Separation of the analytes was performed on Zorbax Eclipse XDB-C8 column (5 μm, 4.6 mm × 150 mm, Agilent, USA). A WGY-I0 Microwave generator (Letter Swan Instrument Co., Changchun, China) with maximum output power of 100 W was used. All solvents used in DUE were degassed before use.

2.2. Chemicals and sample preparation

Water was obtained with a Milli-Q water purification system (Millipore, Bedford, MA, USA). All reagents were of chromatographic reagent grade. Five kinds of *S. baicalensis* Georgi (named as sample 1–5) cultivated in different areas were bought from local drugstores. In the study, all experiments for *S. baicalensis* Georgi were performed on sample 5 except for the experiments mentioned in Section 3.2.3. Flavonoid standards were obtained from National

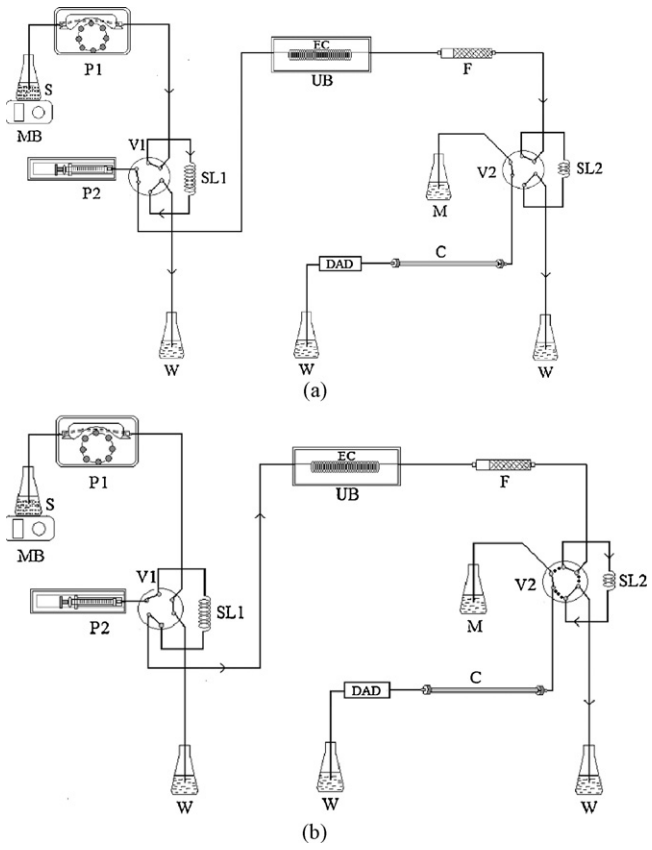

Fig. 2. Schematic diagram of continuous flow-DUE coupled with on-line HPLC system (a) sampling, (b) injection. UB, ultrasonic bath; EC, extraction coil; P1, peristaltic pump; P2, micro-infusion pump; V1 and V2, six-way valve; SL1, sample loop 1; SL2, sample loop 2; MB, magnetic blender; S, mixture of sample and extraction solvent; C, chromatographic column; M, mobile phase; W, waste; F, filter.

Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard stock solutions of flavonoids were prepared in and diluted with methanol.

After being cleaned, the dried roots of *S. baicalensis* Georgi were crushed by FW-100 high-speed disintegrator (Test Instrument Co., Ltd., Tianjin, China) at 12000 r min^{-1} and passed through 80 mesh sieve. The obtained samples were dried thoroughly in the cabinet drier at 40 °C for 48 h. Then the sample powder was stored in the desiccator.

The spiked samples containing baicalin, baicalein and wogonin at concentration of 131.5, 13.6 and 11.0 μg mg^{-1} were prepared by spiking the standard stock solutions into sample powders mentioned above. To ensure the standard solution to be well distributed, a reasonable amount of methanol was added to moisten the sample powder and careful agitation was performed followed by an air-drying for 24 h at ambient temperature before sample analysis.

2.3. Procedure

2.3.1. On-line continuous flow-DUE-HPLC

6 mg of sample was weighed and mixed with 10 mL of extraction solvent by magnetic blender in a flask to obtain the suspension consisting of sample and extraction solvent. The suspension was introduced into the sample loop 1 (SL1, 1 mL) by a peristaltic pump 1 (P1) (Fig. 2a). Then the suspension in the SL1 containing 0.6 mg of sample was delivered by micro-infusion pump (P2) through extraction coil (EC: 320 cm × 3 mm i.d.) located in the ultrasonic bath, then filtrated by the filter at the end of the coil and injected into the sample loop 2 (SL2, 20 μL) of HPLC system (Fig. 2b). Finally, the extract in the SL2 was introduced into the HPLC column.

2.3.2. Off-line continuous flow-DUE

When the continuous flow-DUE was coupled with off-line HPLC system, the procedure was the same as the method above mentioned except that the extract out of the filter was first introduced into the sample bottle and then injected into HPLC.

2.3.3. DMAE, UE and RE

The procedures for DMAE, UE and RE were the same as those described in literature [22]. In DMAE, the flow rate of extraction solvent and the microwave power were set at 2 mL min^{-1} and 80 W, respectively. The other extraction parameters were shown in Table 6. Before analysis by HPLC, the sample solutions obtained by UE and RE were filtered through a 0.45 μm filter membrane.

2.4. Determination of target compounds

2.4.1. Determination of total flavonoids by spectrophotometry

Total flavonoids were determined by UV–Vis spectrophotometry at the wavelength of 280 nm. The standard curve was constructed and used to determine the total flavonoids. Total flavonoids were calculated against baicalin.

2.4.2. Determination of bioactive components by HPLC

The flow rate of the mobile phase was kept at 1 mL min^{-1}. Mobile phase A and B were water and acetonitrile containing 0.1% phosphoric acid, respectively. The gradient conditions were as follows: 0–15 min, 20–30% B; 16–20 min, 30–50% B; 21–28 min, 50–20% B. The temperature of column was controlled at 35 °C. Injection volume was 20 μL. The monitoring wavelength, reference wavelength and bandwidth were 280, 360 and 4 nm, respectively.

3. Results and discussion

3.1. Selection of DUE conditions

In this section, the variables were first studied separately for optimum range, and the most relevant parameters were studied with a orthogonal model. The experimental results in Section 3.1.1 to Section 3.1.5 were based on those obtained by the continuous flow-DUE off-line coupled with spectrophotometric detection for determination of total flavonoids. In Section 3.1.7 the results were obtained based on the determination of baicalin by on-line continuous flow-DUE-HPLC.

3.1.1. Effect of extraction solvent

It is known that flavonoids are compounds containing many hydroxyl groups conferring a high solubility in alcohols. Hence, methanol, ethanol, mixtures of methanol or ethanol–water have been tested. The extraction yield obtained with 80% methanol is higher than that obtained with the 70% ethanol. In our previous study [58], the 60 and 70% ethanol was proved to be most efficient to extract the total flavonoids from the *Scutellaria barbata* D. Don. Besides the difference of target compounds, the reason may be that the viscosity of ethanol (1.074 mPa s at 25 °C) is higher than that of methanol (0.544 mPa s at 25 °C). On the one hand, the acoustic cavitation occurs more easily in the solvent with low viscosity because the molecular forces of the solvent can more easily be exceeded by the ultrasonic intensity applied. On the other hand, the solvent with low viscosity can more easily diffuse into the pores of plant materials [62]. The results also indicate that the extraction yields of total flavonoids first increase and then decrease with the increase of methanol or ethanol concentration. It is clear that the addition of water enhances the extraction yields. The increase of yields in

Table 1
Analysis of L9 $(3)^4$ test result.

No.	(A) Concentration of methanol in extraction solvent	(B) Ultrasonic power	(C) The flow rate of extraction solvent	Extraction yield ($\mu g\,mg^{-1}$)
1	A_1	B_1	C_1	77.08
2	A_1	B_2	C_2	71.64
3	A_1	B_3	C_3	78.89
4	A_2	B_1	C_1	129.07
5	A_2	B_2	C_2	63.75
6	A_2	B_3	C_3	66.84
7	A_3	B_1	C_1	100.81
8	A_3	B_2	C_2	86.11
9	A_3	B_3	C_3	49.15
K1	75.87	102.32	76.68	
K2	85.55	73.83	83.29	
K3	78.69	64.96	81.15	
R	9.68	37.36	6.61	

the presence of water might be due to the increase in swelling of plant material by water, which increases the contact surface area between the plant matrix and the solvent [63]. Additionally, we tried to use water as extraction solvent, the experimental results indicated that water was not suitable because of emulsification of extract and low recovery.

3.1.2. Effect of ultrasonic power

The ultrasonic power is very important to ensure an efficient extraction and effect of this variable was examined. The extraction yields obtained at different power (0, 50, 75, 100 and 150 W) show a rapid increase from 0 to 50 W and a slow increase thereafter. It is clear that, the obtained extraction yield of total flavonoids is very low without ultrasonic irradiation. Hence, the benefit of ultrasonic irradiation in the extraction process is very important.

3.1.3. Effect of solvent flow rate

In most cases, extraction time is the most influential factor on the extraction yield, which often lies on the flow rate and the number of extraction cycles in continuous-dynamic mode. But in the experiment, the sample was passed through the extraction system and introduced into the HPLC system. So there was not the cycle of the sample in the extraction system and the extraction time was related to the flow rate of the extraction solvent. The effect of solvent flow rate ranging from 0.5 to 3.0 mL min^{-1} (corresponding extraction time ranging from 54 to 9 min) on the extraction yields was examined. It was observed that when the solvent flow rate was too high and too low, the extraction yields decreased 14.5 and 18.25%, respectively. The reason is that short irradiation time resulted from the high flow rate makes the ultrasonic extraction of analyte insufficient, and long extraction time resulted from the low flow rate can make the analyte be decomposed.

3.1.4. Effect of amount of sample

When the different amount of sample was introduced into the system, the extraction yields show a platform from 1 to 8 mg. Considering the weighting and determining error, 6 mg was chosen as amount of sample.

3.1.5. Effect of the particle size of sample

To optimize the particle size of sample, 20, 40, 60, 80 and 110 mesh samples were prepared. It was observed that when samples were ground to the size less than 60 mesh, blockage occurred in the extraction tubing. The results also indicated that the extraction yields first increased from 60 to 80 mesh but slowly leveled off from 80 to 110 mesh. When 80 mesh sample was analyzed, the highest extraction yield was obtained.

3.1.6. Orthogonal experiment

Based on the previous experimental results for single-factor, orthogonal experiment (L9 (3^4)) was carried out in order to determine the optimum experimental conditions. The effects of (A) concentration of methanol in extraction solvent (A_1, 70%; A_2, 80%; A_3, 90%), (B) ultrasound power (B_1, 75 W; B_2, 100 W; B_3, 150 W) and (C) flow rate of extraction solvent (C_1, 0.5 mL min^{-1}; C_2, 1.0 mL min^{-1}; C_3, 1.5 mL min^{-1}) on the extraction yields are shown in Table 1. In the table, K_n is the mean effect of each factor at the different levels and R is the range. In this study, the particle size was fixed to 80 mesh and the sample amount was 6 mg. From the table, it can be seen that the (B) ultrasonic power plays an important role in the extraction followed by (A) the concentration of methanol in extraction solvent and (C) the flow rate of extraction solvent. Based on the experimental results, the ultrasonic power, the concentration of methanol in the extraction solvent and the flow rate of extraction solvent were selected as 50%, 80% methanol and 1.0 mL min^{-1}, respectively. Under the selected conditions, the extraction time was 18 min.

3.1.7. Stability of target analytes

To investigate the stability of target analytes, UE was applied. The results indicated that extraction yields of the three flavonoids all show a rapid increase with the increase of extraction time from 0 to 45 min and slightly change from 45 to 60 min. The extraction yield of baicalin slightly decreases with the increase of extraction time from 60 to 90 min and decreases by 8% when the extraction time is 90 min. When the extraction time increases from 60 to 90 min, the decrease of the extraction yields of baicalein and wogonin is not obvious. The experimental results indicate that the analytes are basically stable in the ultrasonic extraction.

3.2. Evaluation of the method

3.2.1. Standard curve and limit of quantification

The baicalin, baicalein and wogonin were determined by continuous flow-DUE coupled with on-line HPLC and the standard curves were constructed by plotting the peak area measured versus the concentration of analyte. Total flavonoids were determined by spectrophotometry and the standard curve was constructed by plotting the absorbance measured versus the concentration of flavonoids. The linear regression equations and correlation coefficients are listed in Table 2. The limits of detection (LODs) and limits of quantification (LOQs) of the methods, indicated in Table 2, were determined as the lowest concentration yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively. The concentrations of the target analytes in the extract are higher than the LOQs and lower than upper limits of determination for the proposed method. So the LOQs and linear equations are appropriate to the goal of the proposed method.

Table 2
Regression equation, LODs and LOQs for total flavonoids, baicalin, baicalein and wogonin.

Coumpound	Regression equation[a]	Correlation coefficient	Liner range ($\mu g\,mL^{-1}$)	LOD ($\mu g\,mL^{-1}$)	LOQ ($\mu g\,mL^{-1}$)
Total flavonoids	$A = 1.93 \times 10^{-3} + 4.68 \times 10^{-2}c$	0.9999	1.69–135.0	0.38	1.26
Baicalin	$A = -33.00 + 57.79c$	0.9996	0.55–109	0.30	1.00
Baicalein	$A = 7.23 + 46.72c$	0.9999	0.51–105	0.37	1.23
Wogonin	$A = 14.93 + 24.21c$	0.9997	0.53–102	0.41	1.37

[a] The number of replicates is 3.

Table 3
The analytical results of samples.

Sample	Method	Baicalin		Baicalein		Wogonin	
		Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, $n = 5$–7)	Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, $n = 5$–7)	Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, $n = 5$–7)
1	On-line	108.3	4.77	6.8	4.57	4.4	4.01
	Off-line	112.8	2.47	6.7	1.34	4.5	3.25
2	On-line	131.5	6.24	13.6	4.44	11.0	5.30
	Off-line	128.4	3.30	13.5	3.72	11.2	4.30
3	On-line	73.8	6.02	15.9	5.36	14.3	4.31
	Off-line	75.0	3.71	15.7	1.25	14.0	1.65
4	On-line	89.0	5.43	15.9	4.20	10.7	3.53
	Off-line	91.8	2.24	15.4	1.63	10.6	3.92
5	On-line	105.9	4.71	11.6	4.33	7.8	3.98
	Off-line	109.1	1.57	11.4	3.18	7.6	3.07

3.2.2. Stability of the system

In the case of solid samples, a small amount of sample will cause problems with sample homogeneity and repeatability of the analytical results. In the study, the repeatability due to suspensions prepared by mixing the sample with the extraction solvent and homogeneity of 10 mL suspension in the flask and 1 mL of suspension in SL1 will affect the precision of the analytical results. In order to examine the effect of preparation of the suspension on the results, five suspensions separately prepared were analyzed by the procedure mentioned in Section 2.3.1 and the experimental results showed that the RSDs for baicalin, baicalein and wogonin are 6.24, 4.44 and 3.30%, respectively. In order to examine the homogeneity of 10 mL of the suspension in the flask, 1 mL of the suspension was introduced into the SL1 at a time and eight replicates were finished by the procedure mentioned in Section 2.3.1. The obtained RSDs for baicalin, baicalein and wogonin are 5.75, 5.21 and 4.43%, respectively. In the experiment, the volume of the suspension in the SL1 is 1 mL but the injection volume of extract in the SL2 is only 20 μL. In order to test the homogeneity of the suspension in the SL1, 1 mL of the suspension was delivered through the extraction coil and separately collected in 10 sample bottles. Then the collected 10 portions of extract were analyzed and the obtained RSDs for baicalin, baicalein and wogonin are 2.44, 2.34 and 3.25%, respectively.

3.2.3. Analysis of samples

To examine the applicability of the proposed method the samples obtained from five different cultivated areas were analyzed. The results (Table 3) indicate that the contents of baicalin, baicalein and wogonin are in the range of 73.8–131.5, 6.7–15.9 and 4.4–14.3 $\mu g\,mg^{-1}$, respectively. For comparison, extraction yields of three flavonoids obtained by continuous flow-DUE coupled with off-line detection are also shown in Table 3 and the differences of the yields obtained by the two methods are not significant. The RSDs obtained by the on-line method are acceptable but poorer than those obtained by the off-line method.

A previous investigation by Horvath et al. [12] was finished by UE using MeOH–water–formic (70:29:1) as extraction solvent and the content of baicalin, baicalein, and wogonin in roots of *S. baicalensis* Georgi were 150, 4.7 and 0.7 $\mu g\,mg^{-1}$ (greenhouse grown tissue), respectively. Lin et al. [64] applied the same method and the obtained extraction yields were 113.5 $\mu g\,mg^{-1}$ for baicalin, 5.7 $\mu g\,mg^{-1}$ for baicalein, and 2.3 $\mu g\,mg^{-1}$ for wogonin. The flavonoid concentrations in *S. baicalensis* roots reported by Zhang et al. [65] were 144 $\mu g\,mg^{-1}$ for baicalin, 29.9 $\mu g\,mg^{-1}$ for baicalein, and 9.7 $\mu g\,mg^{-1}$ for wogonin (dry weight). The differences in flavonoid concentrations reported in those literature were due to the difference in cultivated area, growth conditions and picking period. However, the concentrations of the flavonoids obtained by the proposed method and reported in the literature [64,65] increase in the same order: baicalin ≫ baicalein > wogonin. The analytical results for spiked samples indicate that the present method provides acceptable recoveries (Table 4).

3.2.4. Comparison of different extraction methods

In order to evaluate the performances of the proposed method, other extraction methods were also applied. The chromatogram of the extract obtained by the proposed method is shown in Fig. 3 and not significantly different from those obtained by DMAE, UE and RE. The extraction yields (Table 5) obtained by four methods mentioned above are not significantly different, but the RSDs obtained by the on-line method are poorer than those obtained by the other three methods. The extraction time of the proposed method is only comparable to that of DMAE (3 min) but much shorter than

Table 4
Recoveries of baicalin, baicalein and wogonin from sample 5.

	Original ($\mu g\,mg^{-1}$)	Added ($\mu g\,mg^{-1}$)	Found ($\mu g\,mg^{-1}$)	Recovery (%)	RSD (%, $n = 3$–5)
Baicalin	131.5	130.8	253.4	93.2	6.35
Baicalein	13.6	14.2	25.4	89.9	4.76
Wogonin	11.0	10.5	20.5	90.6	4.97

Table 5
Extraction yields obtained by on-line-DUE, DMAE, UE and SE.

Method	Baicalin		Baicalein		Wogonin	
	Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, n = 3–5)	Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, n = 3–5)	Extraction yield ($\mu g\,mg^{-1}$)	RSD (%, n = 3–5)
On-line-DUE	131.5	6.24	13.6	4.44	11.0	5.30
DMAE	135.2	4.05	13.4	3.75	9.4	1.47
UE	124.8	3.36	14.7	3.67	10.9	4.24
RE	132.6	4.70	13.8	3.22	9.0	2.97

Table 6
Comparison of performances for different extraction methods.

	DUE	DMAE	UE	RE
Extraction mode	Dynamic	Dynamic	Bath	Bath
Extraction time (min)	15	3	60	180
Extraction solvent	80% methanol	60% ethanol	80% ethanol	70% methanol
Solvent volume (mL)	1	6	60	60
Final volume (mL)	1	25	250	250
Injection model	Continuous	Discontinuous	Discontinuous	Discontinuous
Determining model	On-line	Off-line	Off-line	Off-line
On-line filter	Yes	Yes	No	No
Amount of sample (mg)	6	10	100	100

those of UE (60 min) and RE (180 min). Compared with DMAE, in the proposed method, the sample treatment was on-line performed in a closed system. In the experiment, small sample amount was another feature with pros and cons. In fact, too small sample amount may be the reason of poor repeatability. Although the RSDs of the proposed method are acceptable, further improvement is still needed. In our previous study [58], the dynamic ultrasonic extraction (DUE) coupled with on-line detection by spectrophotometer was proposed for the determination of total flavonoids in *S. barbata* D. Don. In that study, continuous determination, monitoring and rapid optimization of the extraction process can be performed conveniently, but the separation and determination of target compounds cannot be achieved at the same time. Yang et al. proposed another method [66], in which dynamic continuous ultrasound-assisted extraction with high intensity ultrasonic probe (CUAE-HIUP) was combined with solid-phase extraction (SPE) for preconcentration and clean-up of the extract prior to high performance liquid chromatographic separation. The method was attractive in combination of CUAE-HIUP and SPE, fast extraction (3 min) and high extraction yield (98.9%), but the on-line detection was not accomplished and HIUP was expensive. Table 6 shows the comparison of performances of the methods mentioned above, and the results indicate that the proposed method is suitable for extracting the target compounds from medicinal plants.

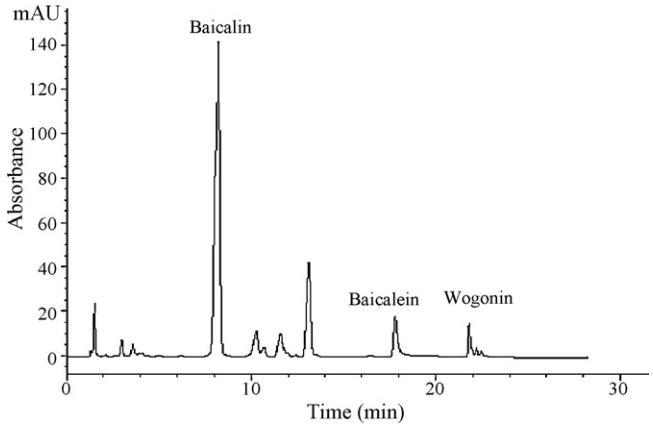

Fig. 3. Chromatogram of extract obtained by continuous flow-DUE coupled with on-line HPLC separation and detection.

4. Conclusion

In this work a continuous sampling DUE coupled with on-line high performance liquid chromatographic separation of the flavonoids in *S. baicalensis* Georgi was developed. The main advantages of the proposed method over traditional techniques include on-line measurement, less consumption of sample and solvent, simple operation and inexpensive extraction set-up. The proposed method was successfully applied to the direct determination of bioactive components in medical plant samples. Besides solid samples, the proposed method can also easily be applied to the liquid samples. The method should be applied to the determination of organic compounds from environmental, biological and food samples.

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