Li-Hong Ye[1]
Xi-De Liu[1]
Jun Cao[2]
Yan-xu Chang[3]*
Mingrui An[4]
Shu-Ling Wang[2]
Jing-Jing Xu[2]
Li-Qing Peng[2]

[1]Department of Traditional Chinese Medicine, Hangzhou Red Cross Hospital, Hangzhou, P. R. China
[2]College of Material Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou, P. R. China
[3]Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, P. R. China
[4]Department of Surgery, University of Michigan Medical Center, Ann Arbor, MI, USA

Received March 12, 2016
Revised August 5, 2016
Accepted August 30, 2016

Research Article

Analysis of isoquinoline alkaloids using chitosan-assisted liquid–solid extraction followed by microemulsion liquid chromatography employing a sub-2-micron particle stationary phase

A simple, efficient, and green chitosan-assisted liquid–solid extraction method was developed for the sample preparation of isoquinoline derivative alkaloids followed by microemulsion LC. The optimized mobile phase consisted of 0.8% w/v of ethyl acetate, 1.0% w/v of SDS, 8.0% w/v of *n*-butanol, 0.1% v/v acetic acid, and 10% v/v ACN. Compared to pharmacopoeia method and organic solvent extraction, this new approach avoided the use of volatile organic solvents, replacing them with relatively small amounts of chitosan. Under the optimum conditions, good linearity ($r^2 > 0.9980$) for all calibration curves and low detection limits between 0.05 and 0.10 μg/mL were achieved. The presented procedure was successfully applied to determine alkaloids in *Rhizoma coptidis* with satisfactory recoveries (81.3–106.4%).

Keywords:
Alkaloids / Chitosan / Liquid–solid extraction / Microemulsion liquid chromatography / *Rhizoma coptidis*
DOI 10.1002/elps.201600114

1 Introduction

Microemulsions (ME) are classified as oil-in-water (O/W) ME, bicontinuous ME, and water-in-oil (W/O) ME, and usually used as the pseudostationary phase in CE [1–5]. In recent years, ME used as a mobile phase in HPLC has been receiving increasing attentions. This separation mode, namely, microemulsion liquid chromatography (MELC), is exhibiting great potential in separation fields [6–9]. Currently, common chromatographic columns, such as Zorbax Extend-C_{18}, Spherisorb C_{18}, and Zorbax-Eclipse XDB-C_8, with particle sizes of 3–5 μm are widely used in conventional MELC [10]. Therefore, establishing an MELC system with sub-2-μm particle size column is quite meaningful in the analysis of complex chemical constituents.

Chitosan (CS) based biomaterials are divided into the following categories based on the range of molecular weight: low-molecular-weight CS, medium-molecular-weight CS, and high-molecular-weight CS [11, 12]. Research efforts have been aimed at tailoring the properties of CS through chemical modification and physical blending via various crosslinking mechanisms, which improved its water solubility (carboxylated CS) and controlled the degree of deacetylation (low-viscosity CS). In the past decades, CS and its derivatives have been extensively used in the fields of cosmetics, food preservation, drug delivery, and environmental protection due to their biocompatibility, nontoxicity, adsorption performance, and biodegradability [13, 14]. At present, CS was usually used as a modification or a component of a composite material in the extraction field [15, 16]. As far as we know, application of a single CS to the sample extraction is scarce and hardly any of the previous articles focused on the application of CS in liquid–solid extraction (LSE).

So far, several techniques have been studied to extract target phytochemicals, including ultrasonic extraction, microwave-assisted extraction, SPE, and accelerated solvent extraction [17–20]. However, these traditional extraction methods possessed several drawbacks, such as the application of large volume of organic solvents (methanol, ACN, acetone, etc.) [21, 22]. It is of great significance to establish a more universal and greener extraction technique to extract the complicated natural products.

Rhizoma coptidis, the dried rhizome of ranunculaceous plants, is commonly used as herbal drugs in China and food additives in other countries. Previously, several methods have been reported for the determination of *R. coptidis* [23–27]. As

Correspondence: Dr. Jun Cao, College of Material Chemistry and Chemical Engineering, Hangzhou Normal University, Hangzhou 310036, P. R. China
E-mail: caojun91@163.com
Fax: +86-571-2886-7909

Abbreviations: CS, chitosan; **LSE**, liquid–solid extraction; **ME**, microemulsions; **MELC**, microemulsion LC; **O/W**, oil-in-water

*Additional corresponding author: Prof. Yan-xu Chang
E-mail: tcmcyx@126.com

Colour Online: See the article online to view Figs. 2, 3 and 4 in colour.

Table 1. Comparison of the proposed method with reported approaches

Methods	Samples	Extraction technique	Instrumental technique	Mobile phase or buffer	Evaluation	Reference
	R. coptidis	UE	CE	Buffer: 60 mM phosphate buffer saline (pH 8.0) with 50% v/v methanol	Requires the use of organic reagents (20 mL of 80% ethanol), large sample amount (1 g), poor detection limit (0.81–4.11 μg/mL)	[23]
	R. coptidis	UE	CE	Buffer: (20:80, v/v) methanol–ACN mixture containing 20 mM sodium acetate solution	Consumes organic reagents (50 mL), requires the use of organic reagents (methanol/HCl (100:1)), high detection limit (0.31–0.34 μg/mL), large sample amount (0.2 g)	[24]
	R. coptidis	Refluxing	LC-MS/MS	Mobile phase: eluent A was water containing 5 mmol ammonium acetate adjusted to pH 5.0 with formic acid, and B was ACN	Large sample amount (1000 g), consumes organic reagents (60% ethanol), long extraction time (2 h)	[25]
	Coptis chinensis Franch	ASE	UPLC	Mobile phase: ACN and 0.50% acetic acid solution with 20 mmol/L ammonium acetate (volume ratio 32:68)	Consumes reagents (50 mL), the consumption of toxic reagents (methanol), large sample amount (1 g)	[26]
Greener alternative	*R. coptidis*	MAE	HPLC	Mobile phase: water (0.4% triethylamine, 20 mM KH_2PO_4, adjusting pH 3 with phosphoric acid)–ACN (60:40, v/v)	Requires large amount of sample (2 g), high limit of detection (2.92–3.99 μg/mL), uses green reagents (aqueous Genapol X-080 solution)	[27]
	R. coptidis	LSE	MELC	Mobile phase: 0.8% w/v of ethyl acetate, 1.0% w/v of SDS, 8.0% w/v of *n*-butanol, 0.1% v/v acetic acid, and 10% v/v ACN	Low detection limit (0.05–0.10 μg/mL), uses green solvent (CS aqueous solution), relatively small sample amount (0.1 g), long dispersion time (60 min) was need for CS	CS assisted LSE

UE, ultrasonic extraction; ASE, accelerated solvent extraction; MAE, microwave-assisted extraction.

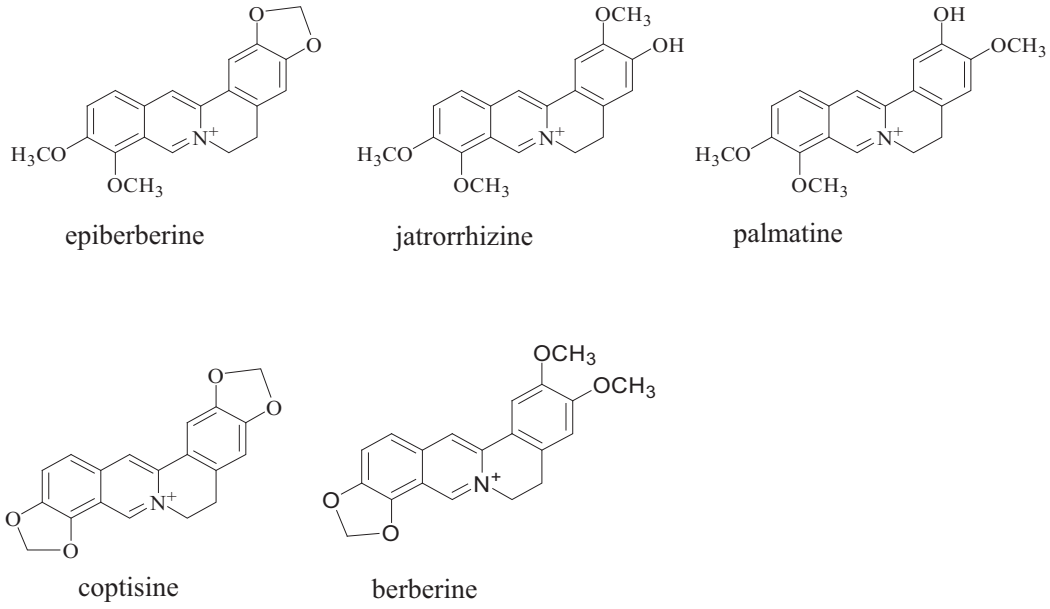

Figure 1. Chemical structures of five isoquinoline derivative alkaloids.

it can be seen in Table 1, these conventional methods such as ultrasonic extraction-CE, refluxing-LC-MS/MS, accelerated solvent extraction-UPLC, and microwave-assisted extraction-HPLC, required larger proportion of the organic phase in the mobile phase or buffer solution. Compared with CS-assisted LSE, the operation of less-green alternative methodologies required the use of organic reagents ((methanol/HCl 100:1), ethanol and methanol) and large sample amount, and consumed large volume of extraction solvents, which did not meet the principles of green chemistry. In addition, the developed technique possessed the merit of less sample amount (0.1 g), and lower detection limit (0.05–0.10 μg/mL) compared with that using aqueous Genapol X-080 solution. The aim of this study was to develop an analytical procedure that combined CS-assisted LSE and MELC, for qualitative and quantitative analyses of alkaloids in *R. coptidis*.

2 Materials and methods

2.1 Chemicals and reagents

Low-viscosity CS (5-20 cp) was obtained from Tokyo Chemical Industry Development Coporation. (Shanghai, China). Carboxylated CS (CS-COOH, water-soluble), low-molecular-weight CS (deacetylation: ≥75.0%, viscosity: 20–300 cp), middle-molecular-weight CS (deacetylation: 75–85%, viscosity: 200–800 cp), and SDS were supplied by Sigma-Aldrich Shanghai Trading Corporation (Shanghai, China). Chromatographic pure *n*-butanol, ethyl acetate, and acetic acid (36-38%) were purchased from Tianjin Siyou Fine Chemical (Tianjin, China). ACN and methanol (HPLC grade) were provided by Merck (Darmstadt, Germany). All other chemicals were of analytical grade. The tested standards of epiberberine, jatrorrhizine, palmatine, coptisine, and berberine were purchased from Shanghai Winherb Medical Technology Corporation (Shanghai, China). The purities of all standards were above 98%. The structures of tested analytes are shown in Fig. 1. Samples of *R. coptidis* were supplied by a local drugstore (Hangzhou, China).

2.2 Instrumentation and chromatographic conditions

The Agilent 1290 series UHPLC system consisted of a binary pump, a thermostated column compartment, a vacuum degasser, and an autosampler (Santa Clara, CA, USA). The ultraviolet wavelength was set at 345 nm and the detector was linked to Agilent Open LAB CDS ChemStation Edition C. 01. 05.

Chromatographic separation was performed using an Agilent reverse-phase SB-C18 column (50 × 4.6 mm id, 1.8 μm particle size). The mobile phase was prepared by weighting 0.8% w/v of ethyl acetate, 1.0% w/v of SDS, 8.0% w/v of *n*-butanol, 0.1 %v/v acetic acid, and 10% v/v ACN, which were then dissolved in 80.1% w/v of water. The mixture was then sonicated for 30 min to aid dissolution. The ME mobile phase was filtered under vacuum through a 0.2 μm filter membrane (a diameter of 50 mm). Sample and the standard solutions of *R. coptidis* were injected into the system and separated at 35°C. The flow rate used in the current study was adjusted to 0.4 mL/min and the injection volume was kept at 1 μL.

2.3 Preparation of standard solutions

Stock standard solutions were prepared by dissolving 0.5 mg of epiberberine, jatrorrhizine, palmatine, coptisine, and berberine in 1 mL of methanol, respectively. The working

standard solutions of five analytes were obtained by diluting appropriate volumes of stock solutions with methanol, and stored at 4°C. All the solutions were filtered through a 0.45 μm nylon membranes before MELC analysis.

2.4 Preparation of sample solutions

2.4.1 Pharmacopoeia method

R. coptidis sample was prepared according to the Chinese Pharmacopoeia 2010 without any modifications [28]. First, *R. coptidis* was comminuted into a homogeneous size by a mill and sieved through a number 100 mesh. Second, the accurately weighed powder (0.2 g) was added to a conical flask, and suspended in 50 mL methanol–HCl (100:1, v/v). Then, the mixture was ultrasonicated at 100 W (40 kHz) for 30 min and the weight loss of the sample solution was compensated with methanol–HCl (100:1, v/v) in the extraction process. After filtering, 2 mL of filtrate was diluted directly to 10 mL with methanol before the chromatographic analysis.

2.4.2 Organic solvent extraction

A total of 0.1 g *R. coptidis* sample was accurately weighed and transferred into a 50 mL conical flask. Then, 20 mL of methanol was added, and the sample was sonicated for 30 min. After centrifugation (13 000 × *g*, 5 min), the supernatant was injected into the UHPLC system.

2.4.3 Preparation of chitosan suspension

Aliquots of 8 mg CS accurately weighed low-viscosity CS, low-molecular-weight CS, and middle-molecular-weight CS were mixed with 20 mL of 1% acidic aqueous solution (adjusting by acetic acid), respectively. The mixture was agitated using an HY-5 cyclotron oscillator for 60 min until it became homogenous. For carboxylated CS, it was directly dispersed in pure water due to the hydrophilic characteristics. The final concentrations were all 0.4 mg/mL.

2.4.4 Chitosan-assisted liquid–solid extraction

The samples of *R. coptidis* were powdered to a homogeneous size in a mill, and passed through a 40-mesh sieve. Then, 0.1 g dried powder was added into 20 mL CS dispersion and the mixture was extracted by sonication for 30 min. The extracts were centrifuged for 5 min at 13 000 × *g*. Finally, the sample solution was directly analyzed by MELC.

2.5 The validation of the method

Mixed standard solutions containing the five alkaloids were diluted to eight different concentration levels for construction of the calibration curves, which were constructed by plotting the peak areas versus the concentrations of the analytes. The intra- and interday precision were tested by analyzing the standard solution at a concentration of 50 μg/mL. The intraday repeatability was determined by assaying the standard mixture six times during one day, and the interday variance was studied for three consecutive days (six analyses). The reproducibility of the method was also assayed by means of repetitive extraction of the *R. coptidis* plant sample ($n = 6$) over a day. The LODs and LOQs were considered as the minimum concentrations of analytes that could be identified and quantified by the methodology, and they were calculated at S/N ratios of 3 and 10, respectively. The recovery study was performed by spiking real samples with the selected standards at two different concentrations (5 and 50 μg/mL in 20 mL of aqueous solutions).

3 Results and discussion

3.1 Optimization of MELC conditions

It is well known that the polarity of the mobile phase in the RP HPLC is relatively larger than that of the stationary phase. It is true that the overall polarity of the O/W ME mobile phase is quite high, that result is due to the solvent (water). Hence, the O/W ME with high aqueous content makes this mobile phase very compatible with the RP chromatography. In MELC, some surfactant molecules adsorb onto the porous RPLC packing and then modify the surface properties of the stationary phase, such as pore volume, surface area, and polarity, which affect drastically chromatographic retention of the solutes and their partition with the stationary phase. In addition, the partitioning mechanism of MELC may relate to interactions among the ME droplets, stationary phase, and aqueous mobile phase, and thus affect their chromatographic performance (Fig. 2). It should be noted that the main issue for MELC is the higher resistance to mass transfer as a result of the reduction in the solute diffusion coefficients in the presence of ME. Therefore, the slow flow rate and the relatively long analysis time were required.

Previous studies showed that the presence of surfactant in ME mobile phase could affect the separation selectivity of target analytes. The effect of SDS concentration on retention time and resolution was investigated in the range of 0.6 to 1.8% w/v. It was found in Fig. 3A that an increase in the SDS concentration decreased the retention time of all the target analytes over the tested range owing to an increased distribution of these compounds into the ME droplets or to the surface of the droplets [29]. However, the separation selectivity was decreased when the concentration of SDS in the mobile phase increased from 0.6 to 1.8%, and analytes 1 and 2 co-eluted into a single peak at 1.4 and 1.8% SDS (Fig. 3A). According to the above observations, 1.0% w/v SDS was used as surfactant in subsequent experiments.

Co-surfactant is usually used to enhance and stabilize the O/W ME. The nature of the co-surfactant influences the phase behavior in the ME system. Experiments with

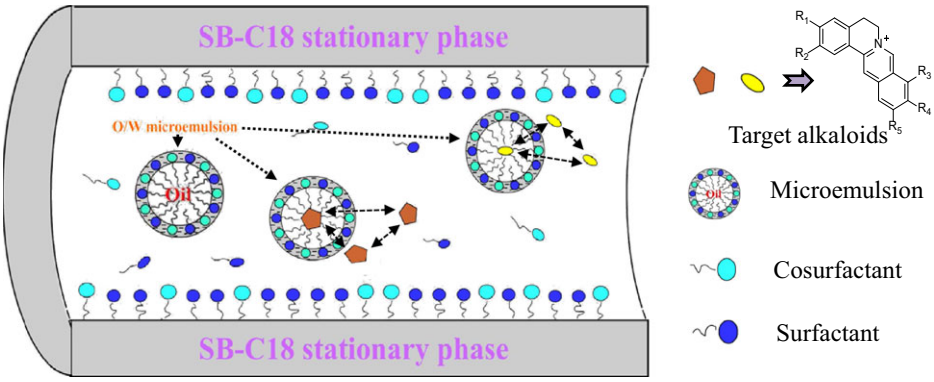

Figure 2. The partitioning mechanism of MELC.

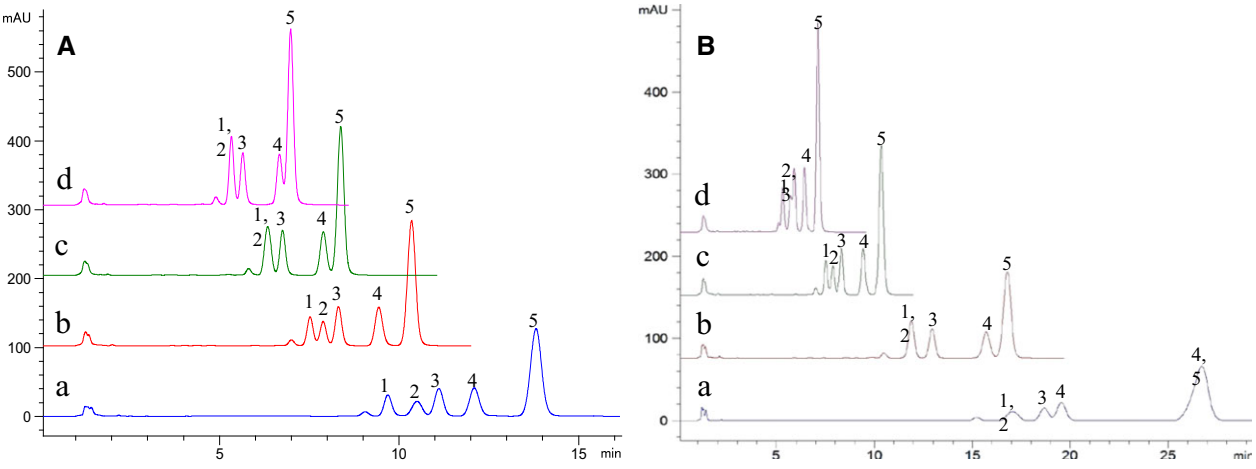

Figure 3. (A) Impact of SDS concentration on the separation of five alkaloids. MELC conditions: 0.8% w/v of ethyl acetate, 8.0% w/v of *n*-butanol, 0.1% v/v acetic acid, and 10% v/v ACN; SDS concentration: (a) 0.6% w/v, (b) 1.0% w/v, (c) 1.4% w/v, (d) 1.8% w/v. Analytes: (1) epiberberine, (2) jatrorrhizine, (3) palmatine, (4) coptisine, (5) berberine. (B) Impact of *n*-butanol concentration on the separation of five alkaloids from *R. coptidis* sample. MELC conditions: 0.8% w/v of ethyl acetate, 1.0% w/v of SDS, 0.1% v/v acetic acid, and 10% v/v ACN; *n*-butanol concentration: (a) 4.0% w/v, (b) 6.0% w/v, (c) 8.0% w/v, (d) 10.0% w/v.

different concentration of *n*-butanol from 4.0 to 10.0% w/v were performed to study its effect on the retention and resolution of the tested compounds. Results showed the retention time decreased noticeably for the five alkaloids as the *n*-butanol concentration increased from 4.0 to 10.0% (Fig. 3B), indicating that the increase of co-surfactant concentration led to an increase in the solubilization capacity of the ME [29]. However, when a very high *n*-butanol concentration was used (10.0%), analytes 2 and 3 overlapped visibly. This is likely due to an increase in the hydrophobicity of the ME with increasing butanol concentration, which may affect retention of tested analytes. In addition, concentrations of less than 8.0% *n*-butanol resulted in broad peaks and reduced sensitivity. Therefore, to obtain the best separation with a short analysis time, 8.0% w/v *n*-butanol was identified as optimal co-surfactant for further work.

Reports have shown that the oil concentration did not significantly affect MELC selectivity [9]. In this study, a slight decrease in retention times of analytes was observed with increasing the oil content from 0.6 to 1.2%. Therefore, 0.8% ethyl acetate was used in subsequent experiments. In MELC, the retention behavior was significantly affected by adding organic solvents. The results showed that the addition of ACN in ME did not affect separation selectivity of analytes, but retention times were decreased. Additionally, acetic acid was used for pH adjustment of ME because the pH value affected the ionization of analytes. It was found that there was no marked effect on the retention of five isoquinoline alkaloids with changing the pH. Based on the experiments discussed above, the optimum ME mobile phase was as follows: 0.8% w/v of ethyl acetate, 1.0% w/v of SDS, 8.0% w/v of *n*-butanol, 0.1 %v/v acetic acid, and 10% v/v ACN.

3.2 Selection of extraction method

3.2.1 Choice of the extraction suspension

The LSE of the analytes from complex samples are related to the properties of the extraction solvents. A suitable extraction solvent should facilitate the transfer of target solutes into solvent through adequate interactions. Thus, different types of CS solvents, including CS-COOH, low-molecular-weight CS, middle-molecular-weight CS, and low-viscosity CS, were

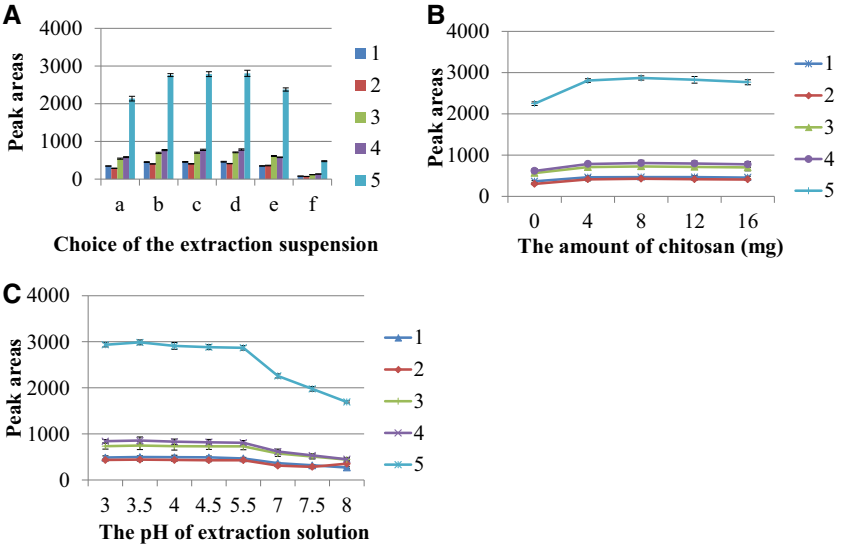

Figure 4. (A) Effect of the extraction suspension on the extraction efficiency of alkaloids. Type: (a) CS-COOH, (b) middle-molecular-weight CS, (c) low-molecular-weight CS, (d) low-viscosity CS, (e) pure methanol, (f) methanol–HCl (100:1, v/v). Analytes: (1) epiberberine, (2) jatrorrhizine, (3) palmatine, (4) coptisine, (5) berberine. (B) Effect of CS amount on the extraction efficiency of alkaloids from *R. coptidis* sample. Extraction conditions: sample amount, 1.0 g; sample volume, 20 mL; CS amount, 0–16 mg. (C) Effect of the solution pH on the extraction efficiency of alkaloids. Extraction conditions: sample amount, 1.0 g; sample volume, 20 mL; CS concentration, 0.4 mg/mL; solution pH, 3–8.

used to evaluate the extraction performance of alkaloids from *R. coptidis* plants. As shown in Fig. 4A(a), the peak areas of the isoquinoline alkaloids were all lower when water-soluble CS-COOH was used as the extraction solvent with regard to the other CS solutions. This finding may be due to the strong hydrophilicity of CS-COOH and hydrophobic groups of the selected compounds, which resulted in a poor interaction between solvent and target compounds. In addition, Fig. 4(b–d) displays that the extraction efficiency was slightly improved with decreasing the CS viscosity (middle-molecular-weight CS: 200–800 cp > low-molecular-weight CS: 20–300 cp > low-viscosity CS: 5–20 cp), demonstrating that the CS with lower viscosity improved the mobility of the aqueous solutions and increased the interface area with plant matrix.

Furthermore, in order to validate the advantages of the proposed approach, a comparison with organic solvent extraction and Chinese pharmacopoeia method [28] was carried out in this work. The results indicated that compared to low-viscosity CS, the extraction yield using methanol or methanol–HCl was poorer (Fig. 4(e and f)). The mechanism of CS-assisted LSE mainly includes the following aspects: the π–π interactions between the aromatic part of the alkaloids and cationic properties of CS; dispersive-type interactions between alkyl groups of the solutes and the side chains of CS; hydrogen-bond interactions between the nonbonding electron pairs of tested alkaloids [30]. It should be indicated that the complete extraction of target alkaloids depended much more on the nature of the cations of CS. Considering the environmental friendliness and extraction efficiency, low-viscosity CS was selected as the best extracting solvent.

3.2.2 Effect of CS amount

The content of CS is a crucial parameter influencing the extraction performance of the tested analytes. Therefore, different amounts of CS ranging from 4 to 16 mg were dissolved in 20 mL acidic aqueous solutions (pH 5.5). Absence of CS meant that 0.1 g of sample was directly extracted by pure water at pH 7.0. The experimental data obtained are shown in Fig. 4B. According to expectations, the peak areas of the five alkaloids were enhanced by increasing the CS amounts from 0 to 8 mg. This aspect might be attributed to the fact that higher number of CS molecules presented a higher interaction with the model compounds as well as increased the kinetics of the extraction procedure. Thus, the extraction efficiency was improved. However, a slight decrease in extraction yield of solutes was observed when the CS amount increased from 12 to 16 mg. A possible reason is that the viscosity of aqueous solution was increased with the increase of CS amount, which influenced the kinetics of analyte–solvent interaction. Consequently, 8 mg of low-viscosity CS was chosen as the optimum quantity for the sample extraction.

3.2.3 Effect of pH

Selection of solution pH is also very important in LSE in order to obtain high extraction efficiency. Therefore, the effect of the pH on the peak areas of the five solutes within the range of 3–8 was tested, under the following conditions (20 mL of aqueous mixture and 8 mg of low-viscosity CS). Acidic solutions were adjusted via acidification with acetic acid, while alkaline sample pH values were obtained using 1 M NaOH. The results are illustrated in Fig. 4C. There were few differences on the peak areas at acidic pH values, but for neutral and alkaline conditions, the extraction yield dramatically decreased.

As pH increased from 3 to 8, the chemical forms of five isoquinoline alkaloids changed from positive ions to nearly neutral, due to the presence of oxygen atoms and quaternary ammonium cationic ions on the structure of molecules, leading to an increasing difficulty in the solute-transfer process. Moreover, neutral and alkaline solutions were not conducive to the dispersion of CS, which decreased the possible hydrophobic, ionic, and hydrogen bond interactions between

Table 2. Linearity, precision, LODs, and LOQs of the target analytes

Analytes	Calibration curves	r^2	Linear range (μg/mL)	Intraday repeatability ($n = 6$)		Interday reproducibility (3 days)		Reproducibility between samples ($n = 6$, RSD%)	LODs (μg/mL)	LOQs (μg/mL)
				Retention time	Peak area	Retention time	Peak area	Content (mg/g)		
Epiberberine	$y = 6.467x - 11.35$	0.9992	0.5–100	0.13	0.21	0.52	1.14	1.75	0.10	0.26
Jatrorrhizine	$y = 11.10x + 8.628$	0.9988	0.5–100	0.13	0.24	0.40	1.21	0.95	0.05	0.15
Palmatine	$y = 10.36x - 9.713$	0.9998	0.5–100	0.11	0.22	0.41	1.09	0.74	0.06	0.17
Coptisine	$y = 8.823x - 9.948$	0.9996	0.5–200	0.17	0.78	0.53	1.48	1.38	0.07	0.25
Berberine	$y = 11.25x - 16.90$	0.9993	0.5–500	0.14	1.02	0.40	1.46	1.94	0.07	0.20

extraction solvent and the selected compounds. As can be seen from Fig. 4C, the highest extraction efficiency for these analytes was observed at pH 3.5. Therefore, pH value of 3.5 was applied as the best value for the extraction solution.

3.3 Method validation

Under optimal conditions, a series of experimental parameters, including linearity, intraday repeatability, interday reproducibility, reproducibility between samples, LODs, and LOQs, were investigated to evaluate the proposed method. As listed in Table 2, satisfactory regression coefficients (r^2) ranging from 0.9988 to 0.9998 were obtained for the five alkaloids in the concentration range of 0.5–500 μg/mL. The precision was evaluated by measuring intra- and interday RSDs. The results are listed in Table 2, the variations expressed by RSD% were less than 1.02% for intraday, and 1.48% for interday. In addition, the extraction reproducibility was analyzed using statistical *t*-test. The results showed that four samples were statistically different at 5% significance level according to the *t*-test. The obtained RSD values for retention time and content were in the range of 0.15–0.29% and 0.74–1.94%, respectively.

Table 3. Quantitative analytical results and recovery

Analytes	The content of *R. Coptidis* (mg/g)	Recovery (%)	
		5 μg/mL	50 μg/mL
Epiberberine	15.70	83.7	81.3
Jatrorrhizine	7.77	96.2	101.4
Palmatine	14.59	89.3	98.6
Coptisine	19.61	100.6	94.9
Berberine	53.40	106.4	90.4

The LOD in chemical analysis is an important parameter for CS-assisted LSE. The lower LOD is very advantageous, especially if the sample concentration is very low. Table 1 shows that the LODs and LOQs for target analytes were found to be 0.05–0.10 and 0.15–0.26 μg/mL, respectively. Considering all validation results, the presented method was accurate and reliable for the determination of alkaloids in *R. coptidis* samples.

3.4 Sample analysis

In order to assess the applicability and reliability of the developed method, it was used to determine five isoquinoline alkaloids in *R. coptidis* plant by MELC. On the basis of the standard curves above, the epiberberine, jatrorrhizine, palmatine, coptisine, and berberine were found at the level of 7.77–53.40 mg/g, as summarized in Table 3.

The data obtained showed that the recovery values were in the interval from 81.3 to 106.4% (Table 3). Figure 3A(b) exhibits the typical chromatogram of *R. coptidis*. The experimental results demonstrated that the proposed approach was a useful extraction tool for the analysis of multiple components in real plant matrices.

4 Concluding remarks

In this study, for the first time, a simple and effective LSE method using CS aqueous solution coupled with MELC was developed for the simultaneous determination of epiberberine, jatrorrhizine, palmatine, coptisine, and berberine in *R. coptidis*. The results indicated that the selected compounds were successfully analyzed with satisfactory repeatability, recovery, and reproducibility. Moreover, compared with other reported approaches, the main advantages of developed method are the simplicity of operation, environmental friendliness, and detection limits at the low-microgram-

per-milliliter level. Therefore, the proposed methodology is promising and can be used for the extraction of other chemical components in the complicated plant samples.

This study was supported by the General Program of National Natural Science Foundation of China (no. 81274065), Zhejiang Provincial Natural Science Foundation of China (LY15H280016), Hangzhou Social Development of Scientific Research Projects (no. 20150533B05), Research on Public Welfare Technology Application Projects of Zhejiang Province (no. 2014C37069), and the Young and Middle-Aged Academic Leaders of Hangzhou (2013-45).

The authors have declared no conflict of interest.

5 References

[1] Schwarze, M., Pogrzeba, T., Volovych, I., Schomäcker, R., *Catal. Sci. Technol.* 2015, *5*, 24–33.

[2] Cao, J., Qu, H. B., Cheng, Y. Y., *Electrophoresis* 2010, *31*, 1689–1696.

[3] Ryan, R., Altria, K., McEvoy, E., Donegan, S., Power, J., *Electrophoresis* 2013, *34*, 159–177.

[4] Cao, J., Chen, J., Yi, L., Li, P., Qi, L. W., *Electrophoresis* 2008, *29*, 2310–2320.

[5] Ryan, R., McEvoy, E., Donegan, S., Power, J., Altria, K., *Electrophoresis* 2011, *32*, 184–201.

[6] Malenović, A., Dotsikas, Y., Mašković, M., Jančić–Stojanović, B., Ivanović, D., Medenica, M., *Microchem. J.* 2011, *99*, 454–460.

[7] Xuan, X. Y., Xu, L. Y., Li, L.X., Gao, C. K., Li, N., *Int. J. Pharmaceut.* 2015, *490*, 258–264.

[8] Mašković, M., Dotsikas, Y., Malenović, A., Jančić–Stojanović, B., Ivanović, D., Medenica, M., *J. AOAC Int.* 2011, *94*, 723–734.

[9] Marsh, A., Clark, B. J., Altria, K. D., *J. Sep. Sci.* 2005, *28*, 2023–2032.

[10] Zhou, J., Zhang, Q., Sun, J. B., Wang, F. Q., Zeng, P., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2014, 951–952, 24–31.

[11] Jayakumar, R., Menon, D., Manzoor, K., Nair, S. V., Tamura, H., *Carbohydr. Polym.* 2010, *82*, 227–232.

[12] Xu, J. J., Ye, L. H., Cao, J., Cao, W., Zhang, Q. Y., *J. Chromatogr. A* 2015, *1409*, 11–18.

[13] Pourjavadi, A., Tehrani, Z. M., Jokar, S., *Polymer* 2015, *76*, 52–61.

[14] Pourjavadi, A., Tehrani, Z. M., Salimi, H., Banazadeh, A., Abedini, N., *Iran. Polym. J.* 2015, *24*, 725–734.

[15] Liao, Q. G., Wang, D. G., Luo, L. G., *Anal. Bioanal. Chem.* 2014, *406*, 7571–7579.

[16] Ziaei, E., Mehdinia, A., Jabbari, A., *Anal. Chim. Acta* 2014, *850*, 49–56.

[17] Hai, N. V., *Aquaculture* 2015, *446*, 88–96.

[18] Cao, W., Yi, L., Cao, J., Hu, S. S., Li, P., *Curr. Drug Metab.* 2014, *15*, 966–987.

[19] Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Dairi, S., Aoun, O., Khodir, M., *Food Chem.* 2015, *187*, 507–516.

[20] Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K., *Food Chem.* 2015, *166*, 585–595.

[21] Zeng H., Wang, Y. Z., Kong, J. H., Nie, C., Yuan, Y., *Talanta* 2010, *83*, 582–590.

[22] Hadi, B., Sanagi, M. M., Ibrahim, W. A. W., Jamil, S., AbdullahiMu'azu, M., Aboul-Enein, H. Y., *Food Anal. Methods* 2015, *8*, 1373–1381.

[23] Xu, X. Q., Wang, J. P., Wen, F. Y., Chen, G. N., *Anal. Methods* 2015, *7*, 976–981.

[24] Hou, J. Y., Li, G., Wei, Y. Q., Lu, H., Jiang, C., Zhou, X. T., Meng, F. Y., Cao, J., Liu, J. X., *J. Chromatogr. A* 2014, *1343*, 174–181.

[25] Liu, G. H., He, W., Cai, H., Sun, X. M., Hou, W. E., Lin, M. N., Xie, Z. Y., Liao, Q. F., *Anal. Methods* 2014, *6*, 2998–3008.

[26] Chen, J. H., Wang, F. M., Liu, J., Lee, F. S. C., Wang, X. R., Yang, H. H., *Anal. Chim. Acta* 2008, *613*, 184–195.

[27] Sun, C., Liu, H. Z., *Anal. Chim. Acta* 2008, *612*, 160–164.

[28] Pharmacopoeia Commission, *Pharmacopoeia of the People's Republic of China*, Vol. 1, China Medical Science and Technology Press, 2010, pp. 285–286.

[29] El-Sherbiny, D. T., El-Enany, N., Belal, F. F., Hansen, S. H., *J. Pharmaceut. Biomed.* 2007, *43*, 1236–1242.

[30] Ruiz-Ángel, M. J., Carda-Broch, S., Torres-Lapasió, J. R., García-Álvarez-Coque, M. C., *J. Chromatogr. A* 2009, *1216*, 1798–1814.

