1 **Analysis of isoquinoline alkaloids using chitosan-assisted liquid-solid**

2 **extraction followed by microemulsion liquid chromatography employing a**

3 **sub-2-micron particle stationary phase**

4

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21 **ABSTRACT**

22 A simple, efficient and green chitosan (CS) assisted liquid-solid extraction method was
23 developed for the sample preparation of isoquinoline derivative alkaloids followed by
24 microemulsion liquid chromatography. The optimized mobile phase consisted of 0.8% w/v of
25 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
26 acetonitrile. Compared to pharmacopoeia method and organic solvent extraction, this new
27 approach avoided the use of volatile organic solvents, replacing them with relatively small
28 amounts of CS. Under the optimum conditions, good linearity (r^2>0.9980) for all calibration
29 curves and low detection limits between 0.05 and 0.10 μg/mL were achieved. The presented
30 procedure was successfully applied to determine alkaloids in *Rhizoma coptidis* with
31 satisfactory recoveries (81.3%-106.4%).

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33 ***Keywords:*** Alkaloids, Chitosan, Liquid-solid extraction, Microemulsion liquid
34 chromatography, Rhizoma coptidis

35 1. Introduction

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

45 Chitosan (CS)-based biomaterials are divided into the following categories based on the
46 range of molecular weight: low-molecular-weight CS, medium-molecular-weight CS and
47 high-molecular-weight CS [11,12]. Research efforts have been aimed at tailoring the
48 properties of CS through chemical modification and physical blending via various
49 crosslinking mechanisms, which improved its water solubility (carboxylated CS) and control
50 the degree of deacetylation (low viscosity CS). In the past decades, CS and its derivatives

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51 have been extensively used in the fields of cosmetics, food preservation, drug delivery and
52 environmental protection due to their biocompatibility, nontoxicity, adsorption performance
53 and biodegradability [13, 14]. At present, CS was usually used as a modification or a
54 component of a composite material in the extraction field [15,16]. As far as we know,
55 application of a single CS to the sample extraction is scarce and hardly any of the previous
56 articles focused on the application of CS in liquid-solid extraction (LSE).

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

63 *Rhizoma coptidis*, the dried rhizome of ranunculaceous plants, is commonly used as
64 herbal drugs in China and food additives in other countries. Previously, several methods have
65 been reported for the determination of *Rhizoma coptidis* [23-27]. As it can be seen in Table 1,
66 these conventional methods such as UE-CE, refluxing-LC-MS/MS, ASE-UPLC and
67 MAE-HPLC, required larger proportion of the organic phase in the mobile phase or buffer
68 solution. Compared with CS assisted LSE, the operation of less green alternative
69 methodologies required the use of organic reagents ((methanol/HCl 100:1), ethanol and

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70 methanol) and large sample amount, and consumed large volume of extraction solvents,

71 which did not meet the principles of green chemistry. In addition, the developed technique

72 possessed the merit of less sample amount (0.1 g), and lower detection limit (0.05-0.10

73 μg/mL) compared with that using aqueous Genapol X-080 solution. The aim of this study

74 was to develop an analytical procedure that combined CS assisted LSE and MELC, for

75 qualitative and quantitative analyses of alkaloids in *Rhizoma coptidis*.

76 **2 Materials and methods**

77 **2.1. Chemicals and reagents**

78 Low viscosity CS (5-20 cp) was obtained from Tokyo Chemical Industry Development Co.,

79 Ltd. (Shanghai, China). Carboxylated CS (CS-COOH, water-soluble), low-molecular-weight

80 CS (deacetylation:≥75.0%, viscosity: 20-300 cp), middle-molecular-weight CS

81 (deacetylation: 75-85%, viscosity: 200-800 cp) and sodium dodecyl sulfate (SDS) were

82 supplied by Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Chromatographic

83 pure n-butanol, ethyl acetate and acetic acid (36-38%) were purchased from Tianjin Siyou

84 Fine Chemical (Tianjin, China). Acetonitrile and methanol (HPLC grade) were provided by

85 Merck (Darmstadt, Germany). All other chemicals were of analytical grade. The tested

86 standards of epiberberine, jatrorrhizine, palmatine, coptisine and berberine were purchased

87 from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). The purities of all

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88 standards were above 98%. The structures of tested analytes are shown in Figure 1. Samples
89 of *Rhizoma coptidis* were supplied by a local drugstore (Hangzhou, China).

90

91 **2.2. Instrumentation and chromatographic conditions**

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

96 Chromatographic separation was performed using an Agilent reverse phase SB-C18
97 column (50 mm × 4.6 mm i.d., 1.8 μm particle size). The mobile phase was prepared by
98 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
99 acetic acid and 10% v/v acetonitrile, which were then dissolved in 80.1% w/v of water. The
100 mixture was then sonicated for 30 min to aid dissolution. The microemulsion mobile phase
101 was filtered under vacuum through a 0.2 μm filter membrane (a diameter of 50 mm). Sample
102 and the standard solutions of *Rhizoma coptidis* were injected into the system and separated at
103 35℃. The flow rate used in the current study was adjusted to 0.4 mL/min and the injection
104 volume was kept at 1 μL.

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106 **2.3. Preparation of Standard solutions**

107 Stock standard solutions were prepared by dissolving 0.5 mg of epiberberine, jatrorrhizine,
108 palmatine, coptisine, and berberine in 1 mL of methanol, respectively. The working
109 standard solutions of five analytes were obtained by diluting appropriate volumes of stock
110 solutions with methanol, and stored at 4 ℃. All the solutions were filtered through a 0.45 μm
111 nylon membranes before MELC analysis.

112

113 **2.4. Preparation of sample solutions.**

114 **2.4.1. Pharmacopoeia method**

115 *Rhizoma coptidis* sample was prepared according to the Chinese Pharmacopoeia 2010
116 without any modifications [28]. First, *Rhizoma coptidis* was comminuted into a homogeneous
117 size by a mill and sieved through a no. 100 mesh. Second, the accurately weighed powder
118 (0.2 g) was added to a conical flask, and suspended in 50 mL methanol-HCl (100:1, v/v).
119 Then, the mixture was ultrasonicated at 100 W (40 kHz) for 30 min and the weight loss of the
120 sample solution was compensated with methanol-HCl (100:1, v/v) in the extraction process.
121 After filtering, 2 mL of filtrate was diluted directly to 10 mL with methanol before the
122 chromatographic analysis.

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123 **2.4.2. Organic solvent extraction**

124 0.1 g *Rhizoma coptidis* sample was accurately weighed and transferred into a 50 mL
125 conical flask. Then, 20 mL of methanol was added, and the sample was sonicated for 30 min.
126 After centrifugation (13,000 rpm, 5 min), the supernatant was injected into the UHPLC
127 system.

128 **2.4.3 Preparation of Chitosan suspension**

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

135 **2.4.4. Chitosan assisted liquid-solid extraction**

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

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140 **2.5 The validation of the method**

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

153

154 **3. Results and discussion**

155 **3.1. Optimization of MELC conditions**

156 It is well known that the polarity of the mobile phase in the reversed phase HPLC is relatively
157 larger than that of the stationary phase. It is true that the overall polarity of the O/W

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158 microemulsion' mobile phase is quite high, that result is due to the solvent (water). Hence,
159 the O/W microemulsion with high aqueous content makes this mobile phase very compatible
160 with the reversed phase chromatography. In MELC, some surfactant molecules adsorb onto
161 the porous RPLC packing and then modify the surface properties of the stationary phase,
162 such as pore volume, surface area and polarity, which affect drastically chromatographic
163 retention of the solutes and their partition with the stationary phase. In addition, the
164 partitioning mechanism of MELC may relate to interactions between the microemulsion
165 droplets, stationary phase and aqueous mobile phase, and thus affect their chromatographic
166 performance (Figure 2). It should be noted that the main issue for MELC is the higher
167 resistance to mass transfer as a result of the reduction in the solute diffusion coefficients in
168 the presence of microemulsions. Therefore, the slow flow rate and the relatively long analysis
169 time were required.

170 Previous studies showed that the presence of surfactant in microemulsion mobile phase
171 could affect the separation selectivity of target analytes. The effect of SDS concentration on
172 retention time and resolution was investigated in the range of 0.6% to 1.8% w/v. It was found
173 in Figure 3A that an increase in the SDS concentration decreased the retention time of all the
174 target analytes over the tested range owing to an increased distribution of these compounds
175 into the microemulsion droplets or to the surface of the droplets [29]. However, the
176 separation selectivity was decreased when the concentration of SDS in the mobile phase

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177 increased from 0.6% to 1.8%, and analytes 1 and 2 coeluted into a single peak at 1.4% and
178 1.8% SDS (Fig. 3A). According to the above observations, 1.0% w/v SDS was used as
179 surfactant in subsequent experiments.

180 Co-surfactant is usually used to enhance and stabilize the O/W microemulsion. The
181 nature of the co-surfactant influences the phase behavior in the microemulsion system.
182 Experiments with different concentration of n-butanol from 4.0% to 10.0% w/v were
183 performed to study its effect on the retention and resolution of the tested compounds. Results
184 showed the retention time decreased noticeably for the five alkaloids as the n-butanol
185 concentration increased from 4.0% to 10.0% (Figure 3B), indicating that the increase of
186 co-surfactant concentration led to an increase in the solubilisation capacity of the
187 microemulsion [29]. However, when a very high n-butanol concentration was used (10.0%),
188 analytes 2 and 3 overlapped visibly. This is likely due to an increase in the hydrophobicity of
189 the microemulsion with increasing butanol concentration, which may affect retention of
190 tested anlytes. In addition, concentrations of less than 8.0% n-butanol resulted in broad peaks
191 and reduced sensitivity. Therefore, to obtain the best separation with a short analysis time,
192 8.0% w/v n-butanol was identified as optimal co-surfactant for further work.

193 Reports have shown that the oil concentration did not significantly affect MELC selectivity
194 [9]. In this study, a slight decrease in retention times of analytes was observed with increasing
195 the oil content from 0.6% to 1.2%. Therefore, 0.8% ethyl acetate was used in subsequent

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196 experiments. In MELC, the retention behaviour was significantly affected by adding organic
197 solvents. The results showed that the addition of acetonitrile in microemusion did not affect
198 separation selectivity of analytes, but retention times were decreased. Additonally, acetic acid
199 was used for pH adjustment of microemulsion because the pH value affected the ionization of
200 analytes. It was found that there was no marked effect on the retention of five isoquinoline
201 alkaloids with changing the pH. Based on the experiments discussed above, the optimum
202 microemulsion mobile phase was as follows: 0.8% w/v of ethyl acetate, 1.0% w/v of SDS,
203 8.0% w/v of n-butanol, 0.1 %v/v acetic acid and 10% v/v acetonitrile.

204

205 **3.2. Selection of extraction method**

206 **3.2.1. Choice of the extraction suspension**

207 The LSE of the analytes from complex samples are related to the properties of the extraction
208 solvents. A suitable extraction solvent should facilitate the transfer of target solutes into
209 solvent through adequate interactions. Thus, different types of CS solvents, including
210 CS-COOH, low-molecular-weight CS, middle-molecular-weight CS and low viscosity CS,
211 were used to evaluate the extraction performance of alkaloids from *Rhizoma coptidis* plants.
212 As shown in Figure 4 Aa, the peak areas of the isoquinoline alkaloids were all lower when
213 water-soluble CS-COOH was used as the extraction solvent with regard to the other CS

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214 solutions. This finding may be due to the strong hydrophilicity of CS-COOH and
215 hydrophobic groups of the selected compounds, which resulted in a poor interaction between
216 solvent and target compounds. In addition, Fig.4 b-d displays that the extraction efficiency
217 was slightly improved with decreasing the CS viscosity (middle-molecular-weight CS:
218 200-800 cp > low-molecular-weight CS: 20-300 cp > low viscosity CS: 5-20 cp),
219 demonstrating that the CS with lower viscosity improved the mobility of the aqueous
220 solutions and increased the interface area with plant matrix.

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

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233 **3.2.2. Effect of CS amount**

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

247

248 **3.2.3. Effect of pH**

249 Selection of solution pH is also very important in LSE in order to obtain high extraction
250 efficiency. Therefore, the effect of the pH on the peak areas of the five solutes within the

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251 range of 3–8 was tested, under the following conditions (20 mL of aqueous mixture and 8 mg
252 of low viscosity CS). Acidic solutions were adjusted via acidification with acetic acid while
253 alkaline sample pH values were obtained using 1 M NaOH. The results are illustrated in
254 Figure 4C. There were few differences on the peak areas at acidic pH values, but for neutral
255 and alkaline conditions, the extraction yield dramatically decreased.

256 As pH increased from 3 to 8, the chemical forms of five isoquinoline alkaloids changed
257 from positive ions to nearly neutral, due to the presence of oxygen atoms and quaternary
258 ammonium cationic ions on the structure of molecules, leading to an increasing difficulty in
259 the solute-transfer process. Moreover, neutral and alkaline solutions were not conducive to
260 the dispersion of CS, which decreased the possible hydrophobic, ionic and hydrogen bond
261 interactions between extraction solvent and the selected compounds. As can be seen from
262 Fig. 4C, the highest extraction efficiency for these analytes was observed at pH 3.5.
263 Therefore, pH value of 3.5 was applied as the best value for the extraction solution.

264

265 **3.3. Method validation**

266 Under optimal conditions, a series of experimental parameters, including linearity, intra-day
267 repeatability, inter-day reproducibility, reproducibility between samples, limits of detection
268 (LODs), and limits of quantification (LOQs), were investigated to evaluate the proposed

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269 method. As listed in Table 1, satisfactory regression coefficients (r^2) ranging from 0.9988 to
270 0.9998 were obtained for the five alkaloids in the concentration range of 0.5–500 μg/mL. The
271 precision was evaluated by measuring intra- and interday RSDs. The results are listed in
272 Table 2, the variations expressed by relative standard deviation (RSD%) were less than
273 1.02% for intra-day, and 1.48% for inter-day. In addition, the extraction reproducibility was
274 analyzed using statistical t-test. The reaults showed that four samples were statistically
275 different at 5% significance level according to the t-test. The obtained RSD values for
276 retention time and content were in the range of 0.15%-0.29% and 0.74%-1.94%, respectively.

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

282

283 **3.4. Sample analysis**

284 In order to assess the applicability and reliability of the developed method, it was used to
285 determine five isoquinoline alkaloids in *Rhizoma coptidis* plant by MELC.

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286 On the basis of the standard curves above, the epiberberine, jatrorrhizine, palmatine, coptisine
287 and berberine were found at the level of 7.77-53.40 mg/g, as summarized in Table 2.

288 The data obtained showed that the recovery values were in the interval from 81.3% to
289 106.4%. Fig. 3A-b exhibits the typical chromatogram of *Rhizoma coptidis*. The experimental
290 results demonstrated that the proposed approach was a useful extraction tool for the analysis
291 of multiple components in real plant matrices.

292

293 **4. Conclusions**

294 In this study, for the first time, a simple and effective LSE method using CS aqueous solution
295 coupled with MELC was developed for the simultaneous determination of epiberberine,
296 jatrorrhizine, palmatine, coptisine and berberine in *Rhizoma coptidis*. The results indicated
297 that the selected compounds were successfully analyzed with satisfactory repeatability,
298 recovery, and reproducibility. Moreover, compared with other reported approaches, the main
299 advantages of developed method are the simplicity of operation, environmental friendliness,
300 and detection limits at the low μg/mL level. Therefore, the proposed methodology is
301 promising and can be used for the extraction of other chemical components in the
302 complicated plant samples.

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Legends

Figure 1. Chemical structures of five isoquinoline derivative alkaloids.

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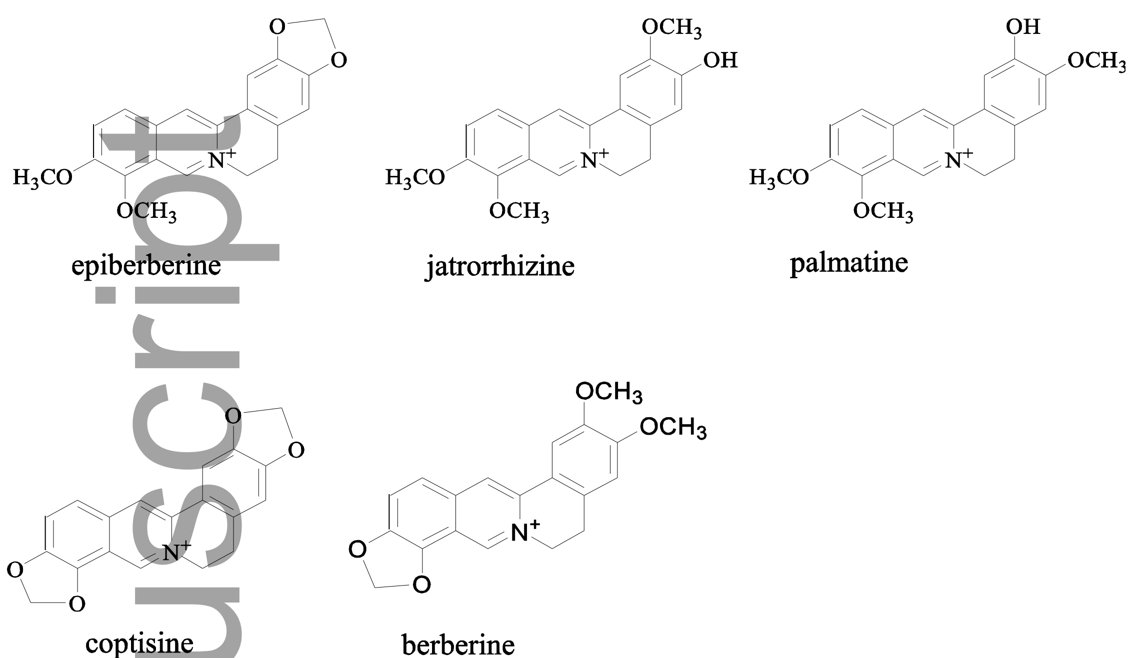

Figure 2. The partitioning mechanism of MELC.

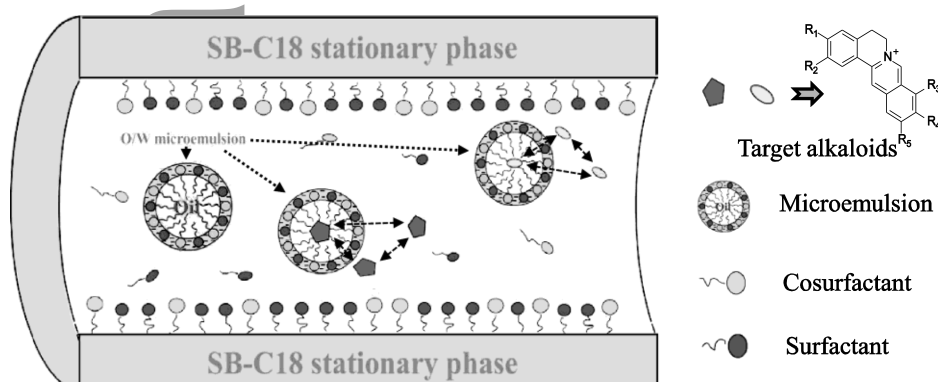

Figure 3. (A) Impact of SDS concentration on the separation of five alkaloids**.** MELC conditions: 0.8% w/v of ethyl acetate, 8.0% w/v of n-butanol, 0.1% v/v acetic acid and 10% v/v acetonitrile, SDS concentration: (a) 0.6% w/v, (b) 1.0%w/v, (c) 1.4%w/v, (d) 1.8%w/v. Analytes: (1) epiberberine, (2)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

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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 acetonitrile, n-butanol concentration: (a) 4.0% w/v, (b) 6.0% w/v, (c) 8.0% w/v, (d) 10.0% w/v.

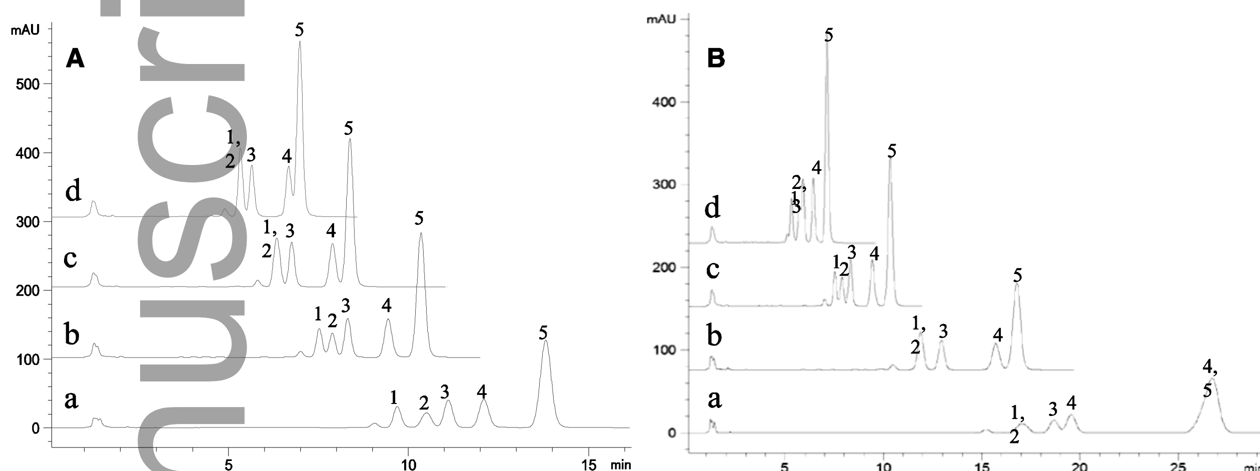

Figure 4. (A) Effect of the extraction suspension on the extraction efficiency of alkaloids. ~~Type:~~ (a) CS-COOH, (b) middle-molecular-weight CS, (c) low-molecular-weight CS, (d) low viscosity CS, (e) pure methanol, (f) methanol-HCl (100:1, v/v). Analytes: (1) epiberberine, (2) jatrorrhizine, (3) palmatine, (4) coptisine, (5) berberine. (B) Effect of CS amount on the extraction efficiency of alkaloids from *Rhizoma 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; sulution pH, 3-8.

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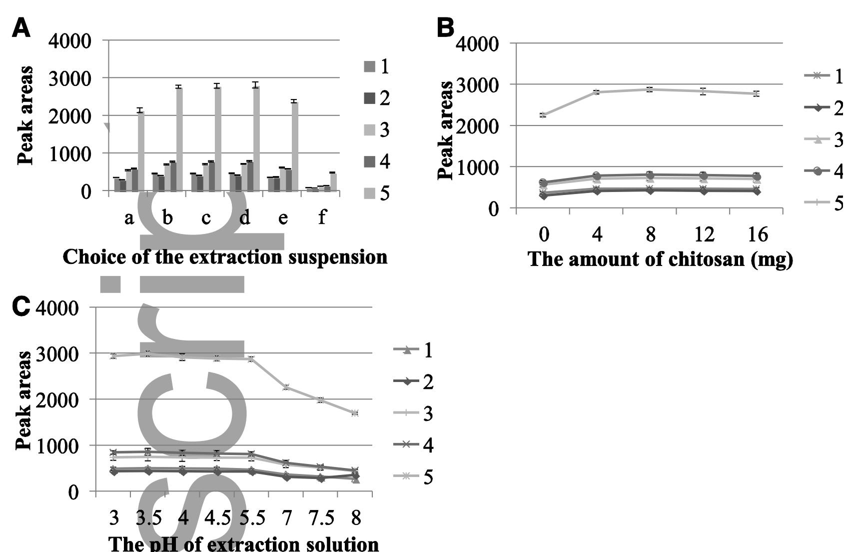

Table 1. Comparison of the proposed method with reported approaches.

s	Extraction technique[a]	Instrumental technique[b]	Mobile phase or buffer	Evaluation
a 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 80% ethanol), large sample amount (1 g), p detection limit (0.81-4.11 μg/mL).
a Coptidis	UE	CE	Buffer: (20:80, v/v) methanol-acetonitrile mixture containing 20 mM sodium acetate solution	Consumes organic reagents (50 mL), requir use of organic reagents (methanol/HCl (100 high detection limit (0.31-0.34 μg/mL), larg sample amount (0.2 g).
a 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 acetonitrile	Large sample amount (1000 g), Consumes reagents (60% ethanol), long extraction tim
chinensis	ASE	UPLC	Mobile phase: acetonitrile and 0.50% acetic	Consumes reagents (50 mL), the consumpti

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			acid solution with 20 mmol/L ammonium acetate (volume ratio 32:68)	toxic reagents (methanol), large sample am g).
a Coptidis	MAE	HPLC	Mobile phase: water (0.4% triethylamine, 20 mM KH_2PO_4, adjusting pH 3 with phosphoric acid)-acetonitrile (60:40, v/v)	Requires large amount of sample (2 g), higl of detection (2.92-3.99 μg/mL), uses green reagents (aqueous Genapol X-080 solution)
a Coptidis	Liquid-solid extraction	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 acetonitrile	Low detection limit (0.05-0.10 μg/mL), use solvent (chitosan aqueous solution), relative small sample amount (0.1 g), long dispersic (60 min) was need for CS

[a]Extraction technique: UE, ultrasonic extraction; ASE, accelerated solvent extraction; MAE, microwave-assisted extraction.

[b]Instrumental technique: CE, capillary electrophoresis; LC-MS/MS, liquid chromatography-tandem mass spectrometry; HPLC, High-performance liquid chromatography; UPLC, ultra performance liquid chromatography; MELC, microemulsion liquid chromatography.

Table 2. Linearity, precision, limits of detection (LODs), and limits of quantification (LOQs) of the target analytes.

Analytes	Calibration curves	r^2	Linear range (μg/mL)	Intra-day repeatability (n=6)		Inter-day reproducibility (3 days)		Reproducibil between samples (n= RSD%)
				Retention time	Peak area	Retention time	Peak area	Content (mg
Epiberberine	y = 6.467 x - 11.35	0.9992	0.5-100	0.13	0.21	0.52	1.14	1.75
Jatrorrhizine	y = 11.10 x + 8.628	0.9988	0.5-100	0.13	0.24	0.40	1.21	0.95

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Palmatine	y = 10.36 x - 9.713	0.9998	0.5-100	0.11	0.22	0.41	1.09	0.74
Coptisine	y = 8.823 x - 9.948	0.9996	0.5-200	0.17	0.78	0.53	1.48	1.38
Berberine	y = 11.25 x - 16.90	0.9993	0.5-500	0.14	1.02	0.40	1.46	1.94

Table 3. Quantitative analytical results and recovery.

Analytes	The content of *Rhizoma 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

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