Microwave-assisted aqueous two-phase extraction of alkaloids from *Radix Sophorae Tonkinensis* with ethanol/Na_2HPO_4 system: process optimization, composition identification and quantification analysis

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Abstract: A rapid method for simultaneous extraction and separation of multiple alkaloids from *Radix Sophorae Tonkinensis* (RST) was developed by microwave-assisted aqueous two-phase extraction (MAATPE) using the aqueous two-phase extraction system (ATPS) of ethanol/Na_2HPO_4 as extraction solvent. The effects of key factors on MAATPE extraction of alkaloids from RST were investigated by single-factor experiment and response surface methodology (RSM). The optimum conditions were concluded as follows: the phase ratio of 2.60 for the ATPS, the particle size of 100 mesh, the liquid-to-material ratio of 75:1, extraction temperature of 90 °C and extraction time of 5 min. The RSM model was significant ($p<0.0001$) and adequate for prediction of process efficacy, and the experimental yield of 29.41±0.21 mg/g for alkaloids under optimum conditions was very close to the predicted value. Compared to conventional extraction methods, alkaloids were extracted preferentially from RST to top phase by MAATPE with higher yield and shorter extraction time. By means of high-resolution ultra-performance liquid chromatography-quadrupole-orbitrap mass spectrometry (UPLC-Q-Orbitrap/MS) and HPLC with UV detection, identification and quantification of nine alkaloids extracted were accomplished. Matrine, sophocarpine, oxymatrin, sophoranol, oxysophocarpine, 5α-hydroxysophocarpine, sophoridine, cytisine and N-methylcytisine in RST were successively determined in range of 0.493–10.284 mg/g with RSD's of 0.8–2.1%. Moreover, MAATPE mechanism was explored by means of the different extraction systems and scanning electron microscopy. Significant differences in extraction yield and cell rupture exhibited that the addition of the salt in the mixed solvents not only improved thermal effect and demixing effect, but also accelerated the mass transfer process and biphasic extraction capacity. MAATPE integrated the advantages of microwave-assisted extraction (MAE) and aqueous two-phase extraction (ATPE). Hence, it was also proved as a green, efficient and promising alternative to

extraction of alkaloids from RST.

Keywords: Microwave-assisted aqueous two-phase extraction; Response surface methodology; *Radix Sophorae Tonkinensis*; alkaloids; HPLC analysis; UPLC-orbitrap-MS/MS

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1. Introduction

Radix Sophorae tonkinensis (RST) is the dried roots and rhizomes of *Sophora tonkinensis* Gapnep., also named Shandougen, has been commonly used as a traditional Chinese medicine for the treatment of detoxification and alleviation of pain (Chinese Pharmacopoeia Commission, 2015a; Chen et al., 2017). Quinolizidine alkaloids as the main beneficial components in RST have been demonstrated the pharmacological effects such as anti-inflammation, anti-tumor, and antiviral activities, etc (Chen et al., 2017; Han et al., 2016; Pan et al., 2015; Tang et al., 2013). In addition, some matrine-type alkaloids have exhibited insecticidal effects for green pesticides in agriculture (Ma et al., 2018, Villaverde, et al., 2016; Xiong, et al., 2016; Zanardi, et al., 2015). So far, more and more alkaloids including matrine-type and cytisine-type have been extracted from RST in succession for further development of biological activities (Ding et al., 2006; Zhang et al., 2016b). Also, cytisine-type alkaloids were reported to have shown multi-pharmacological effects of the neuron protection and antidepressant property, etc (Li et al., 2013; Rouden et al., 2014). Recently, toxic side effects of the alkaloids have attracted more and more attention due to adverse clinical cases (Chen, et al., 2017; Liu, et al., 2017; Tian, 2016). For pharmaceutical uses, RST is one of the most important sources of quinolizidine alkaloids (especially matrine and oxymatrine as primary ingredients in pharmaceutical preparations) besides *Sophora flavescens* Ait. (Chinese Pharmacopoeia Commission, 2015b).

At present, alkaloids in RST are usually extracted by cold-merceration, heating reflux extraction, liquid membrane extraction, ultrasonic-assisted extraction (UAE) and accelerated solvent extraction (ASE) using single-phase solvent (Guo et al., 2016; Meng et al., 2013; Sheng and Zhou, 2008; Tang et al., 2013; Wang et al., 2017; Yang et al., 2012). However, these extraction methods are subjected to remarkable shortcomings including lengthy process, high cost of organic solvents, low extraction yield with high impurities, and degradation of the unstable compound except for UAE and ASE with shorter extraction time.

Since aqueous two-phase extraction (ATPE) was first introduced by Albertson (Albertson, 1986), it has been widely used in the separation of biomolecules such as proteins, enzymes, and antibiotics due to simple operation, mild condition, friendly environment, ease of scaling-up and low costs (Glyk et al., 2015; Iqbal et al., 2016; Molino et al., 2013; Ruiz-Ruiz et al., 2012; Soares et al., 2015). An aqueous two-phase systems (ATPS) is usually consisted of two or more phase-forming substances in water (e.g., two polymers, a polymer and a salt, two or more surfactants) under a certain critical condition (Iqbal et al., 2016; Molino et al., 2013; Soares et al., 2015). Since short chain alcohols/hydrophilic solvents came in to use, these ATPSs have been applied to extraction and

purification of lots of compounds from natural products owing to efficient mass transfer, low viscosity and interfacial tension, biocompatibility and nontoxicity (Liu et al., 2013; Soares et al., 2015; Tatjana and Mirjana, 2017; Xu et al., 2017; Zhang et al., 2013;). Recently, a combination technology of microwave-assisted extraction (MAE) and ATPE, known as microwave-assisted aqueous two-phase extraction (MAATPE), has been paid more attentions due to advantages of quick heating, less solvent consumption, low energy expenditure and decrease pollution, etc (Chen et al., 2016; Cheng et al., 2017; Ma et al., 2013; Wang et al., 2008; Xie et al., 2017). Based on biphasic extraction capacity and microwave field intensification, MAATPE can produce the great thermal effect and the rapid demixing effect because microwave can strongly interact with a polar ATPS. As a result, MAATPE integrating extraction and purification processes into one-step procedure, can not only improve extraction efficiency, but also remove coexisted impurities. In our previous work, we observed that the herbs with roots or rhizomes in a polar medium were susceptible to microwave radiation. The interaction of microwave field with an ATPS led to cell rupture and achieved higher extraction yield (Cheng et al., 2017; Xie et al., 2017; Zhang et al., 2015). Thus, MAATPE as a combination of MAE and ATPE is a potential and powerful alternative to the conventional approaches for extraction alkaloids from RST. To our knowledge, there hasn't been any related report so far.

In this study, MAATPE was utilized for extraction and separation of multiple alkaloids from RST with an ATPS of ethanol/disodium hydrogen phosphate system. By means of a closely pressurized microwave system, MAATPE conditions, including screening of the ATPS, the phase ratio of the ATPS, extraction temperature and time, particle size, and liquid-to-material ratio, were investigated by single-factor tests, respectively. Subsequently, key factors were further optimized by response surface methodology (RSM) to improve extraction efficiency. Accordingly, characterization of alkaloids' composition in the extract was qualitatively conducted by UPLC-Q-Orbitrap/MS. Finally, quantification of multiple alkaloids in RST was carried out by high performance liquid chromatography (HPLC) with UV detection. MAATPE mechanism was explored by the different extraction medium and scanning electron microscopy for biphasic property, extraction process and herb surface structure.

2. Materials and methods

2.1. Materials and reagents

Crude drug of RST was obtained from Guangxi province in China, and identified by a botany professor in School of Traditional Chinese Medicine at Guangdong Pharmaceutical University in addition to comparison with the pharmacopoeia (Chinese Pharmacopoeia Commission, 2015a). After being dried and ground into a fine powder, the samples were sieved (40–200 mesh) and placed in desiccators at room temperature. The moisture of RST

powder was determined as 10.82±0.09% (w/w) by measuring the weight difference between before and after drying a given sample according to Chinese Pharmacopoeia Commission.

Oxymatrine and matrine (purities were ≥98.0%) were purchased from Xi'an Xuhuang Bio-Tech Co., Ltd. (China). Oxysophocarpine, sophocarpine and sophoridine (purities ≥98.0%) were bought from Shanghai Pureone Biotechnology Co., Ltd. (China). Cytisine and N-methylcytisine were obtained from Chengdu Herbpurify Co., Ltd. (China). Bromothymol blue (Tianxin Fine Chemical Co., Ltd. China); Acetonitrile and methanol (HPLC grade, Merck Darmstadt Ltd., Germany); All other chemicals were analytical grade (Guangzhou Chemical Reagent Factory, China).

2.2. *Preparation of ATPS*

Phase diagram of ATPS was first prepared by turbidity titration method at a certain temperature (Zhang et al., 2015). A certain amounts of salts (NaH_2PO_4, Na_2HPO_4, Na_3PO_4) were dissolved in deionized water, respectively. Ethanol was subsequently added dropwise into the salt solution until it became turbid. The concentrations of both ethanol and salt at every level were recorded accurately to acquire enough data. Then phase diagrams were plotted by different ethanol concentrations versus ammonium sulfate concentrations at different turbid points.

An ATPS of ethanol/phosphate system was further prepared according to the phase diagram plotted (See Fig. 1). A certain amount of phosphate was dissolved in deionized water by heating in a water bath, and then added ethanol, mixed them by a vortex stirrer. The ATPS was formed when the mixture separated two phases at the cloud point.

2.3. *MAATPE procedure*

All MAATPE experiments were performed on an EXCEL microwave extraction system (PreeKem Scientific Instruments Co., Ltd., China) equipped with a digital timer, power and temperature controller. 0.5 g of sample (100 mesh) and 30 mL of the ATPS (ethanol of 35.85% (w/w) and Na_2HPO_4 of 15.38% (w/w)) were added into an extraction vessel. After sealed, the vessel was placed in the microwave extraction system for extraction of alkaloids at 90 °C for 5 min. The extract was immediately filtered to remove the herb residue. The top phase was collected when two phases were separated.

2.4. *UV–vis analysis*

Total alkaloids in the extract were determined by bromothymol blue method with matrine as standard according to following procedure. 5 mL of phosphate buffer (KH_2PO_4-NaOH, pH=7.0) and 5 mL of 0.12 % bromothymol blue solution (w/w) were added to 1.0 mL of sample solution from top phase. After mixed well, let

the mixture stand for 3 min. Then 10 mL of chloroform was added for extraction of ion-associates produced by alkaloids, and then standing until chloroform layer appeared. After discarded aqueous phase, the extract obtained was detected at 417 nm on a 2550 ultraviolet spectrophotometer (Shimadzu, Japan) against reagent blank. Similarly, matrine standard solutions were also determined according to the above procedure, and then the calibration curve was plotted for calculation of the content of total alkaloids (Regression equation is A (absorbance) =50.914C (Concentration, μg/mL) + 0.0085, R^2=0.9990). The extraction performance was evaluated by the yield (*Y*) of total alkaloids, which were calculated as follows:

$$Yield(mg/g) = \frac{m_{Alkaloids}}{m} \quad (1)$$

Where $m_{Alkaloids}$ is the total amount of alkaloids extracted from the herb powder (*m*).

2.5. Experimental design and statistical analysis

RSM is a widely employed technique in research for designing, evaluating and optimizing for improving processes (Amit, et al., 2016; Bezerra et al., 2008; Yari and Rashnoo, 2017). A four variable, three levels of central composite design (CCD) was applied to optimize key factors for improvement of alkaloids yield. The range and center points of four factors (Phase ratio X_1 of 2.25–2.75, Particle size X_2 of 80–120 mesh, Liquid-to-material ratio X_3 of 65:1–75:1 and extraction temperature X_4 of 80–90°C) were based on the results of single-factor experiments. The total numbers (*N*) of required experiments were calculated according to the equation $N = k^2 + 2k + c_p$ (Here *k* is the factor number and c_p is the replicate number of the central point). The factorial points located at high level (+1) and low level (-1) from the center of the experimental domain. α value of axial points for the CCD was determined by the equation of $\alpha = 2^{k/4}$. In present study, *k* and c_p were set at 4 and 6, respectively, indicating that 30 experiments had to be carried out. The responses for designed experiments were also given in Table 1, and all data were determined by UV–vis analysis in triplicate. Subsequently, the regression analysis and the RSM optimization were completed by Design-Expert software version 8.0. Experimental data were fitted to a quadratic polynomial model and the model was explained by the following quadratic equation:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (2)$$

Where *Y* is the response function, X_i, X_j are the independent variables. β_0 is the intercept, β_i, β_{ii} and β_{ij} are the coefficients of the linear, quadratic and interaction term, respectively.

Table 1 Central composite design (CCD) and the experimental results: phase ratio (X_1), particle size (X_2), liquid-to-material ratio (X_3)

147 and extraction temperature (X_4).

No.	Factors				Extraction yield
	X_1 (V_{Top}/V_{Bottom})	X_2 (mesh)	X_3 (mL: g)	X_4 (°C)	(mg/g)
1	-1 (2.25)	-1 (80)	-1 (65:1)	-1 (85)	22.19±0.24
2	1 (2.75)	-1 (80)	-1 (65:1)	-1 (85)	25.01±0.13
3	-1(2.25)	1 (120)	-1 (65:1)	-1 (85)	22.71±0.30
4	1 (2.75)	1 (120)	-1 (65:1)	-1 (85)	25.77±0.32
5	-1 (2.25)	-1 (80)	1 (75:1)	-1 (85)	24.43±0.38
6	1 (2.75)	-1 (80)	1 (75:1)	-1 (85)	27.73±0.23
7	-1 (2.25)	1 (120)	1 (75:1)	-1 (85)	23.87±0.34
8	1 (2.75)	1 (120)	1 (75:1)	-1 (85)	26.86±0.14
9	-1 (2.25)	-1 (80)	-1 (65:1)	1 (95)	23.51±0.41
10	1 (2.75)	-1 (80)	-1 (65:1)	1 (95)	25.96±0.17
11	-1 (2.25)	1 (120)	-1 (65:1)	1 (95)	24.74±0.59
12	1 (2.75)	1 (120)	-1 (65:1)	1 (95)	25.99±0.35
13	-1 (2.25)	-1 (80)	1 (75:1)	1 (95)	26.47±0.23
14	1 (2.75)	-1 (80)	1 (75:1)	1 (95)	27.75±0.18
15	-1 (2.25)	1 (120)	1 (75:1)	1 (95)	25.99±0.62
16	1 (2.75)	1 (120)	1 (75:1)	1 (95)	26.60±0.51
17	-2 (2.00)	0 (100)	0 (70:1)	0 (90)	21.67±0.33
18	2 (3.00)	0 (100)	0 (70:1)	0 (90)	27.21±0.24
19	0 (2.50)	-2 (60)	0 (70:1)	0 (90)	24.38±0.54
20	0 (2.50)	2 (140)	0 (70:1)	0 (90)	23.87±0.62
21	0 (2.50)	0 (100)	-2 (60:1)	0 (90)	24.96±0.23
22	0 (2.50)	0 (100)	2 (80:1)	0 (90)	28.57±0.39
23	0 (2.50)	0 (100)	0 (70:1)	-2 (80)	24.11±0.63
24	0 (2.50)	0 (100)	0 (70:1)	2 (100)	26.39±0.21
25	0 (2.50)	0 (100)	0 (70:1)	0 (90)	29.09±0.29
26	0 (2.50)	0 (100)	0 (70:1)	0 (90)	28.97±0.35

27	0 (2.50)	0 (100)	0 (70:1)	0 (90)	29.05±0.26
28	0 (2.50)	0 (100)	0 (70:1)	0 (90)	28.79±0.42
29	0 (2.50)	0 (100)	0 (70:1)	0 (90)	28.55±0.54
30	0 (2.50)	0 (100)	0 (70:1)	0 (90)	28.81±0.19

2.6. UPLC–Q-Orbitrap/MS identification of alkaloids

The extract collected from the top phase was pretreated for chromatographic analysis according to the clean-up procedure described by the literature (Zhang et al., 2017). The resulting residue was dissolved and diluted accurately to 5 mL with methanol. After filtered through a 0.22 μm nylon membrane, the sample solution was sealed and kept at 4°C. Then, identification of alkaloids was carried out by using an UPLC–ESI–Q-Orbitrap system (Thermo Fisher Scientific Inc. USA), which is composed of DIONEX UltiMate 3000 UPLC chromatograph coupled with Q Exactive™ Hybrid Quadrupole- Orbitrap Mass Spectrometer, equipped with an autosampler and an electrospray ionization (ESI) source According to the following conditions (Zhang, et al., 2017).

Chromatographic separation was performed on a Phenomenex Kinetex C_{18} column (100 mm×2.1 mm, 1.7 μm). The mobile phase consisted of acetonitrile (A) and 0.01mol/L ammonium acetate with pH=8.0 (B). Alkaloids were eluted at 40℃ in the gradient elution as follows: 0–3.5 min, 9% A; 3.5–4.5 min, 9–20% A; 4.5–10 min, 20% A; 10–12 min, 20–25% A; 12–15 min, 25–9% A. The injection volume was 3 μL and the flow rate of the mobile phase was 0.3 mL/min.

The ESI interface was used in positive ion mode (ESI+) with the following settings. The spray voltage was 3.2 kV. The capillary temperature was set at 320℃. S-lens RF level was set to 50. Aux gas heater temperature was 350℃. Normalised collision energy (NCE) was controlled between 50 and 65%. The flow rate of sheath gas and aux gas were respectively 40, 10 arbitrary units. The Full-MS ranges from 50 to 600 m/z in the orbitrap, with the resolution set at 70000. In our Full-MS-dd-MS^2 (MS-data dependent tandem MS^2), resolution, isolation width and AGC values were respectively set at 17500, 2 m/z, and 2×10^5. An extraction window of 5 ppm was set to obtain extracted ion chromatograms (EICs) of all analytes. The maximum inject times for all MS/MS mode were 100 ms. Xcalibur 3.0 software was used for data acquisition and processing.

2.7. HPLC-UV analysis

Quantification of alkaloids in RST was performed on a 1200 Agilent Infinity chromatograph with UV detector (Agilent Technologies Co., Ltd., USA). All alkaloids extracted were determined by detecting at 220 nm using a Phenomenex Geminin C_{18} Column (5 μm, 250 mm×4.6 mm) as the stationary phase (Zhang, et al., 2015). The

mobile phase was made of methanol (A), acetonitrile (B) and 0.1% (w/v) ammonia solution (C). Alkaloids were eluted at 30°C in the following gradient mode: 0–6 min: 8.5–9.0% A, 9.5–9.0% B and 82% C; 6–11 min: 9.0–16.0% A, 9.0–16.0% B and 82.0–68.0% C; 11–45 min: 16.0% A, 16.0% B, 68.0% C; 45–50 min: 16.0–8.5% A, 16.0–9.5% B and 68.0–82.0% C; 50–55 min: 8.5% A, 9.5% B, 82.0% C. The injection volume was 20 μL. The flow rate was 1.0 mL/min.

2.8. Method validation

Validation of the HPLC-UV method for quantification of alkaloids in RST was performed by testing linearity, precision and recovery. A series of mixed standard solutions at different concentration prepared by diluting the stock solutions (1 mg/mL) with methanol were injected to the chromatograph in turn for HPLC analysis, the data was collected for plotting calibration curves. For repeatability and accuracy, alkaloids in the extracts and the extracts spiked with standards were also determined in 5 and 3 replicates, respectively.

3. Results and discussion

3.1. Screening of the ATPS

Based on the phase separation ability of phosphates versus organic solvents, three ATPSs made of ethanol/NaH_2PO_4, ethanol/Na_2HPO_4 and ethanol/Na_3PO_4 were investigated by the formation of their phase diagrams. The results revealed that the salt with higher valence state is easier to form an ATPS, implying wilder two-phase zone as shown in Fig. 1 (a). However, the solubility of Na_3PO_4 is too low to increase its salt concentration, otherwise salting-out occur. Thus, an ATPS of ethanol/Na_3PO_4 can be formed only at more than 50°C, and the limited phase-forming range led to difficulty in practice. In order to screen out a suitable ATPS for efficient extraction of alkaloids from RST, three ATPSs were used to evaluate their extraction performance at 80°C of extraction temperature under the same extraction conditions. Considering the effect of the composition of an ATPS on the phase ratio (i.e. the ratio of top volume to bottom volume), which was too big or too small to practically operate. Thus, the phase ratio of 1:1 was used for screening experiment. From Fig. 1 (b), most of alkaloids were extracted to the top ethanol-rich phase. Relatively, ethanol/Na_2HPO_4 system had a higher yield of total alkaloids compared to the other two systems, so it was chosen as an extractant for further investigation by comprehensive consideration.

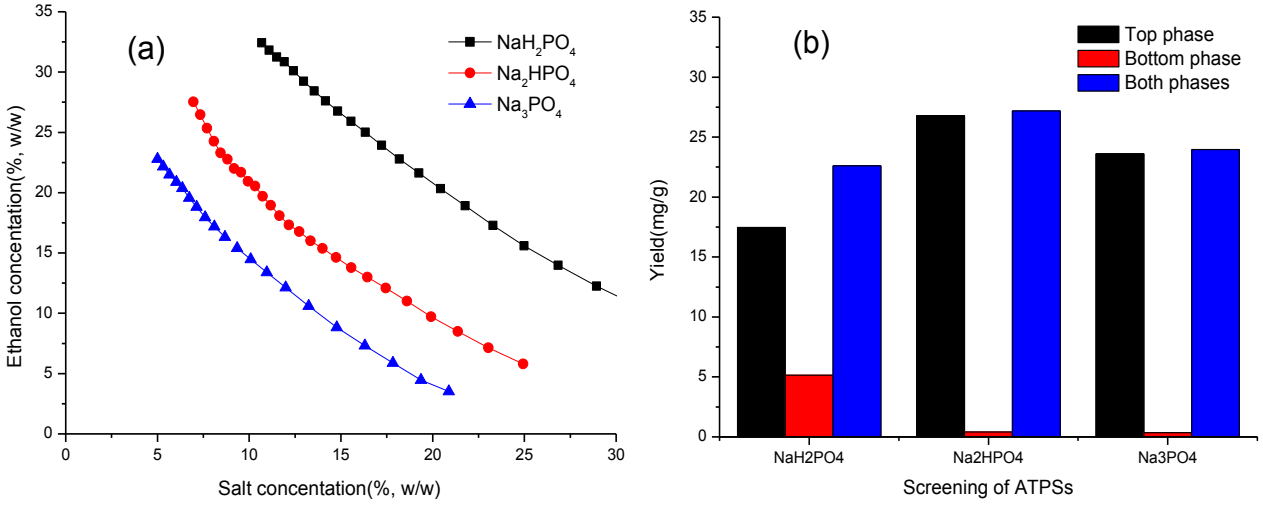


Fig. 1. The phase diagrams at 60 °C (a) and screening of ethanol/phosphate systems (b) according to following conditions: ATPS volume of 30 mL; extraction temperature of 80 °C; extraction time of 10 min; phase ratio of V_{Top}/V_{Bottom} =1:1 (ethanol/NaH_2PO_4 system: NaH_2PO_4 concentration of 32.19% (w/w), ethanol concentration of 27.25% (w/w); ethanol/Na_2HPO_4 system: Na_2HPO_4 concentration of 25.40% (w/w), ethanol concentration of 18.52% (w/w); ethanol/Na_3PO_4 system: Na_3PO_4 concentration of 17.50% (w/w), ethanol concentration of 15.99% (w/w)).

3.2. *Single-factor experiment*

In MAATPE process, the factors affecting the extraction yield of alkaloids were mainly from the phase ratio and volume of an ATPS, the radiation level of the microwave and the state of the material. In order to get high extraction yield of alkaloids using the ATPS, the optimization tests need to be performed. In this study, five major parameters including phase ratio, liquid-to-material ratio, particle size, extraction temperature and time were preliminarily investigated by single-factor experiment using a closely pressurized microwave system.

3.2.1. *The effect of phase ratio*

In fact, extraction performance of alkaloids strongly depends on the phase ratio of the ATPS which is controlled by its composition of both ethanol and Na_2HPO_4. For a certain concentration of the salt solution, increasing the volume of top phase meant to decrease the volume of the bottom phase while total volume of the ATPS kept constant. However, too small volume of the bottom phase would lead to the interface blurring while the herb material exists. In order to investigate the effect of the phase ratio on the extraction yield of alkaloids, experiments were performed at different phase ratios. The results as showed in Fig. 2 demonstrated that alkaloids yield was improved with increase of the phase ratio, then kept relatively stable in range of 2.0–3.5. In fact, alkaloids in RST were preferentially extracted to the top phase, increasing the phase ratio largely facilitated to

improve extraction yield. The maximum yield was at 2.5, implying that the composition of ethanol and water in the top phase had an impact on the extraction performance. Thus, the selection of 2.5 phase ratio is relatively moderate for the following research.

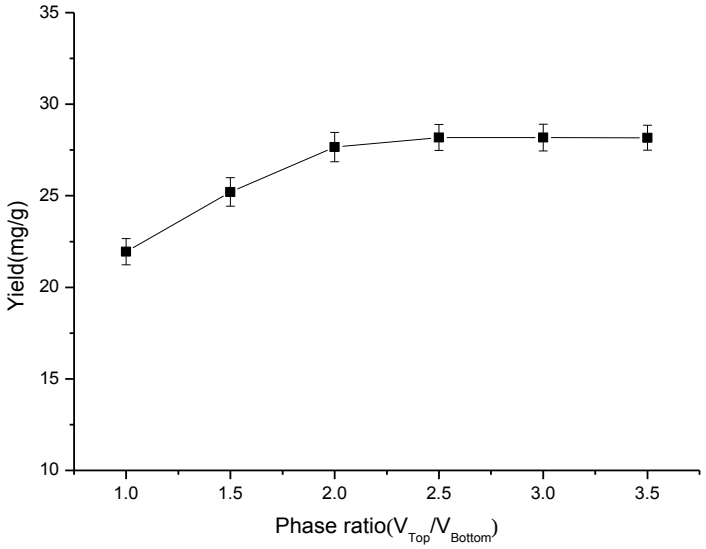


Fig. 2. Effect of phase ratio on the yield of total alkaloids.

3.2.2. The effect of liquid-to-material ratio

Fig. 3 illustrated that the effect of the liquid-to-material ratio (40:1–80:1) on MAATPE extraction of alkaloids from RST. The results showed that alkaloids yield went up gradually when increased the liquid-to-material ratio. The maximum yield was reached at 70:1. The liquid-to-materials ratio was set at 70:1 in next experiments.

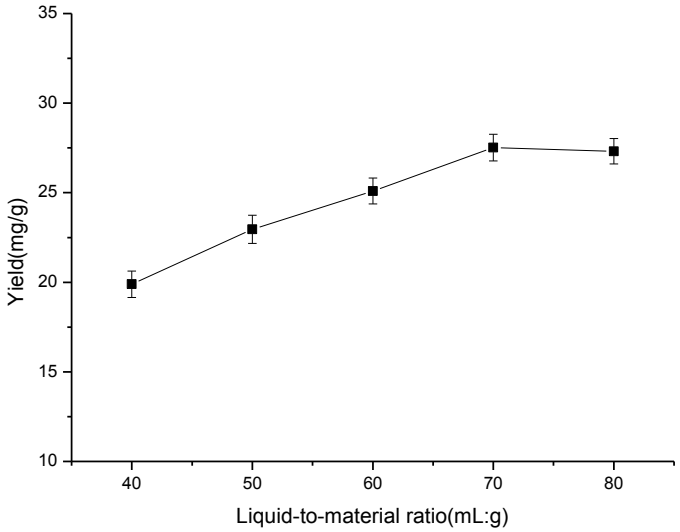


Fig. 3. Effect of liquid-to-material ratio on the yield of total alkaloids.

3.2.3. The effect of particle size

As shown in Fig. 4, the effect of particle size on the extraction of alkaloids was investigated in the range of

40-120 mesh. The results showed that alkaloids yield increased remarkably while reducing particle size. But when particle size was larger than 100 mesh, the amount of alkaloids extracted from RST decreased obviously. In fact, finer particles would make the extract to become more viscous, which limited release of alkaloids from the sample matrix. According to the maximum yield, 100 mesh of particle size was chosen for further study.

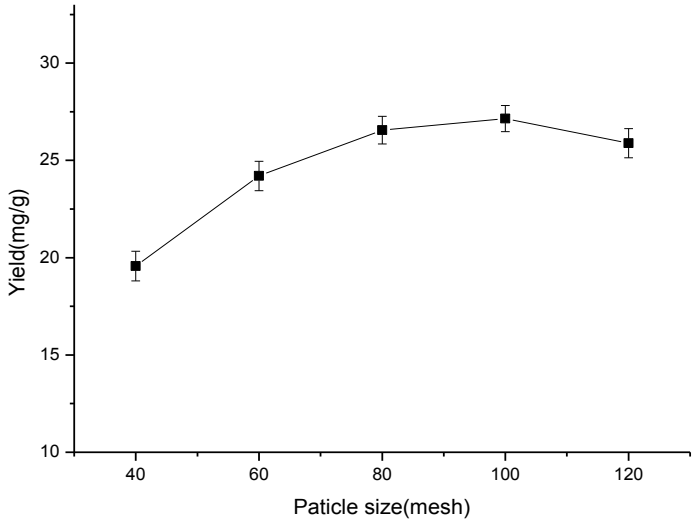


Fig. 4. Effect of particle size on the yield of total alkaloids.

3.2.4. The effect of extraction temperature and time

In the MAATPE process, extraction temperature and time are two key factors affecting extraction of alkaloids from the herb material. In fact, extraction temperature was controlled by the microwave power linking a temperature probe, and depended on the extent of interaction between microwave and the ATPS, so both factors dictated together alkaloids yield. Hence, experiments were carried out at different temperature (60–100°C) and times (2–20 min) to determine more proper parameters. From the results as shown in Fig. 5, alkaloids yield was improved with the increase of extraction temperature in range of 60–90°C then declined, while extraction time of 5 min reached maximum yield. Higher temperature and longer extraction time may lead to decomposition or transformation, and decrease alkaloids yield, especially some quinolizidine alkaloids like oxymatrine that are susceptible to high temperature (Pan, et al., 2008). Extraction of alkaloids from *Sophora flavescens* Ait. containing relatively high amounts of oxymatrine also showed the similar results (Wang, et al., 2012b; Zhang, et al., 2015). Thus, choosing 5 min at 90°C was suitable for extraction of alkaloids in experiments.

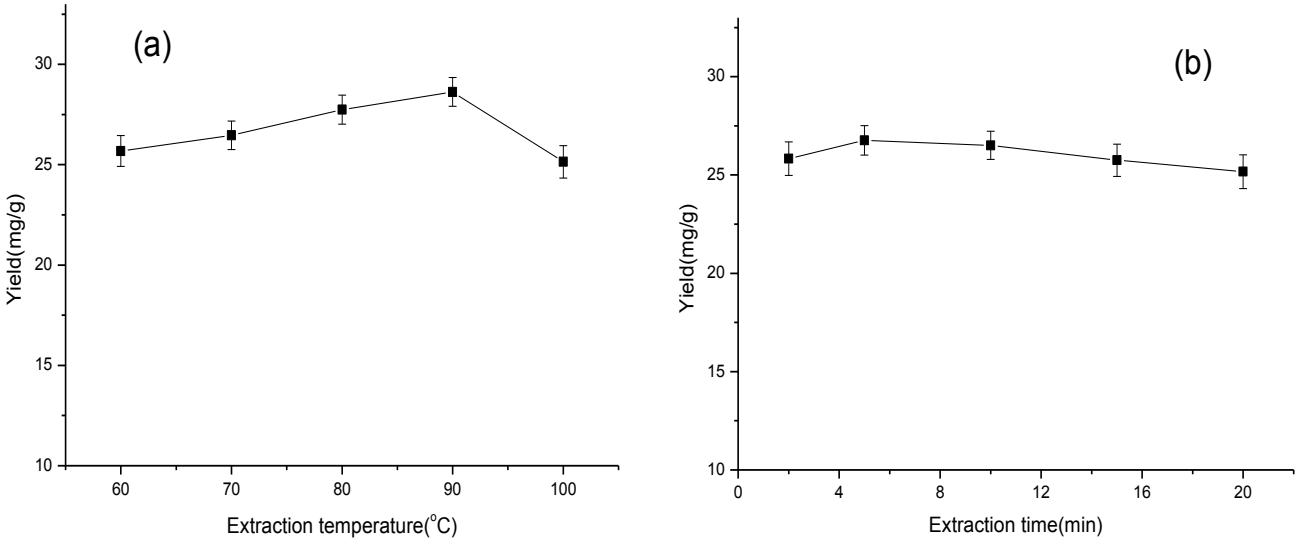


Fig. 5. Effects of extraction temperature and time on the yield of total alkaloids.

On the basis of the above results, optimum conditions for extraction of alkaloids from RST were summarized as follows: the phase ratio of 2.5 for the ATPS, 70:1 of the liquid-to-material ratio, 100 mesh of the particle size, 90°C of temperature and 5 min of extraction time.

3.3. Optimization of MAATPE conditions by response surface methodology

The effects of various factors on alkaloids yield were examined through single-factor experiment, leaving room for further improvement and verification. In particular, interactional relations between some factors needed to be further investigated, so phase ratio, particle size, liquid-to-material ratio and extraction temperature were confirmed as critical influencing factors for RSM optimization. Based on preliminary ranges of the influencing factors determined by the single-factor experiment, optimization of MAATPE process was performed by RSM according to CCD procedure.

3.3.1. Statistical analysis and model fitting

For RSM optimization of MAATPE process, a full factorial design followed with a rotatable central composite design (RCCD) was applied to evaluate the effects of parameters and their interactions (Amit, et al., 2016; Bezerra et al., 2008; Yari and Rashnoo, 2017). Based on the results mentioned above in Table 1, RSM analysis was completed statistically by using 8.0 Design-Expert software to determinate coefficients of the intercept, linear, quadratic, and interaction terms of the model. Analysis of variance (ANOVA) followed by F-test was applied to estimate the significance of each term coupled with the associated p-value. The calculated results of ANOVA were listed in Table 2, and then the fitted regression model was obtained and derived by following equation:

$$Yield\ (mg/g)=28.96+1.15X_1+0.048X_2+0.88X_3+0.57X_4-0.097X_1X_2-0.07X_1X_3-0.36X_1X_4-0.37X_2X_3+0.00875X_2X_4 -0.029X_3X_4-0.84X_1^2-1.22X_2^2-0.55X_3^2-0.93X_4^2 \quad (3)$$

Table 2 Analysis of variance (ANOVA) for the fitted model.

Source	Sum of squares	df	Mean square	F-Value	p-Value*
Model	135.70	14	9.69	98.31	< 0.0001
X_1	34.66	1	34.66	351.50	< 0.0001
X_2	0.099	1	0.099	1.00	0.3326
X_3	18.45	1	18.45	187.08	< 0.0001
X_4	7.04	1	7.04	71.42	< 0.0001
$X_1 X_2$	0.24	1	0.24	2.39	0.1433
$X_1 X_3$	0.12	1	0.12	1.24	0.2825
$X_1 X_4$	2.71	1	2.71	27.45	0.0001
$X_2 X_3$	1.96	1	1.96	19.88	0.0005
$X_2 X_4$	0.00303	1	0.00303	0.031	0.8633
$X_3 X_4$	0.023	1	0.023	0.23	0.6397
X_1^2	31.71	1	31.71	321.61	< 0.0001
X_2^2	36.52	1	36.52	370.45	< 0.0001
X_3^2	6.69	1	6.69	67.88	< 0.0001
X_4^2	20.89	1	20.89	211.88	< 0.0001
Residual	1.48	15	0.099		
Lack of fit	1.28	10	0.13	3.14	0.1090
Pure Error	0.20	5	0.041		
Correct Total	137.18	29			
R-Squared	0.9892				
Adjusted R-square	0.9792				
Predicted R-square	0.9443				
Adequate precision	32.306				

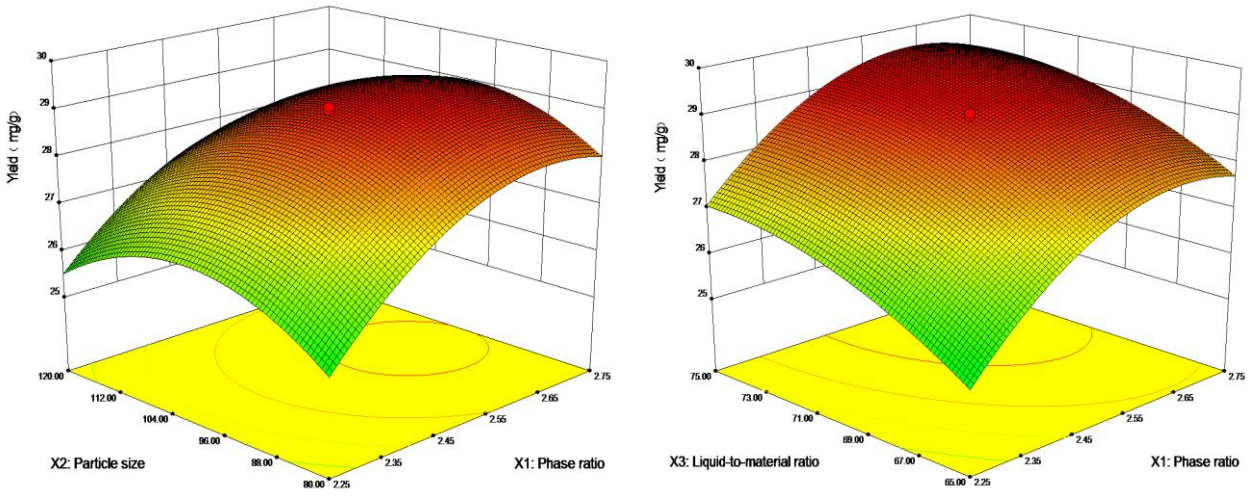
* Very significant ($p<0.01$); significant ($p<0.05$).

The ANOVA results could evaluate whether the regression model was established successfully according to the p-value. The p-value of the model was lower than 0.0001, demonstrating that the model accurately fitted the

relationship between the extraction yield and four key factors mentioned above. The results of error analysis also indicated that the lack of fit was insignificant ($p>0.05$). Moreover, the higher determination coefficients (R^2=0.9892, adjusted R^2=0.9792 and predicted R^2=0.9443) of the model showed the good agreement between predicted and experimental data, and also suggested high suitability for good representation and correlation of the response to variables. The p-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. Seen from Table 2, the linear coefficients (X_1, X_3 and X_4), quadratic coefficients (X_1^2, X_2^2, X_3^2 and X_4^2) were significant factors, and interaction term coefficients (X_1X_4 and X_2X_3) were very significant with very smaller p-values ($p<0.0005$). The other terms coefficients (X_2, X_1X_2, X_1X_3, X_2X_4 and X_3X_4) were not significant ($p>0.05$). Relatively, Phase ratio (X_1) and interaction term (X_1X_4) demonstrated outstanding effect on the extraction yield, implying that the strong interaction of composition of the ATPS and microwave field in the MAATPE process.

3.3.2. Optimization of extraction conditions

For optimization of extraction conditions, the visualization of the model obtained by response surface plots is the best way to investigate the effects of the independent variables on the dependent variable. Generally, generating response surface plots were done by varying two variables within the experimental range and holding the others are constant at the central level (Bezerra et al., 2008). Thus, three-dimensional response surface curves were plotted to visualize the relationship between responses and interaction variables for the maximum yield. The effects of phase ratio (X_1), particle size (X_2), liquid-to-material ratio (X_3) and extraction temperature (X_4) on the extraction yield were presented in Fig. 6 (a)-(f).



(a) (b)

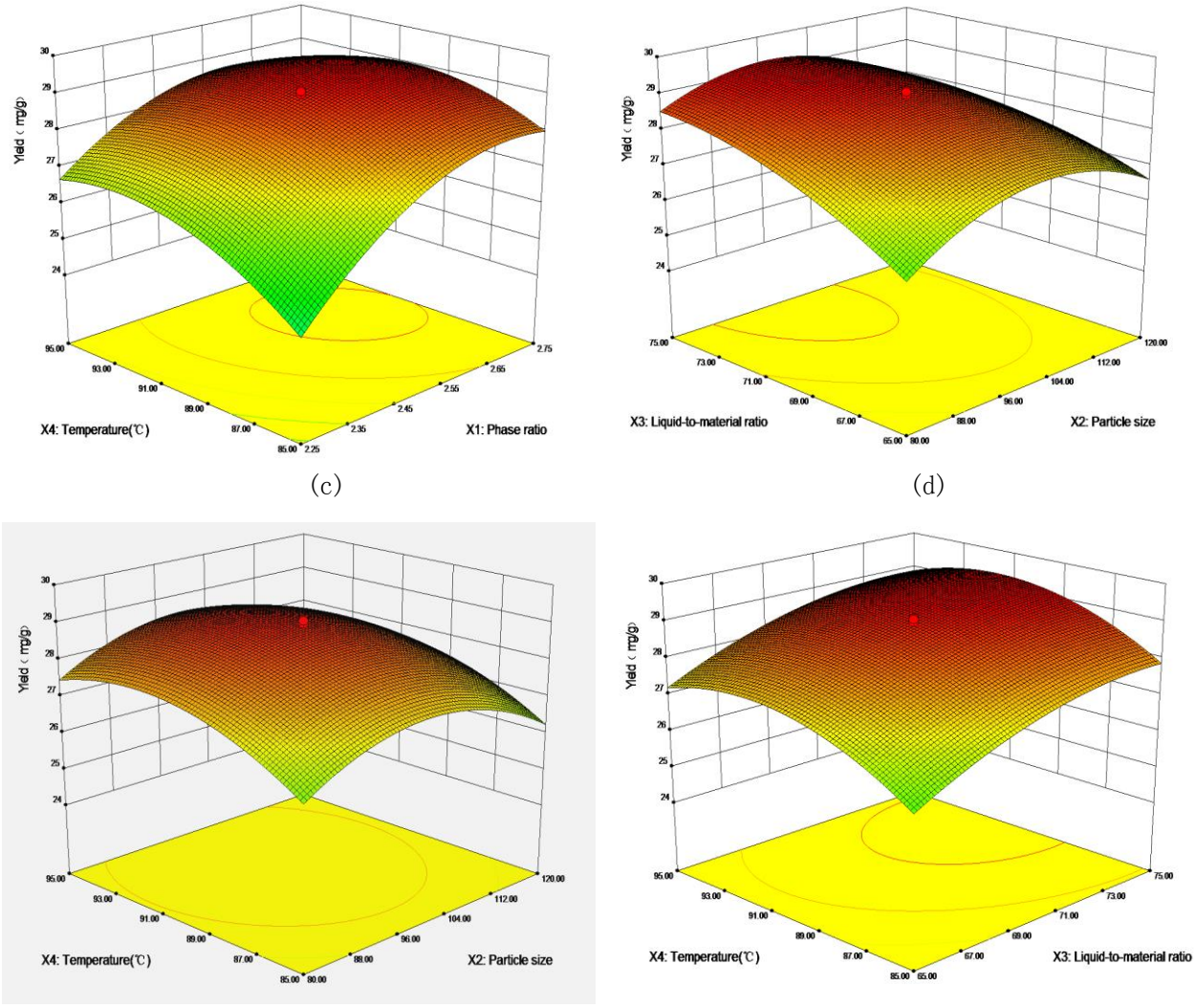


Fig. 6. Response surface curves showing the effects of phase ratio (X_1), particle size (X_2), liquid-to-material ratio (X_3), extraction temperature (X_4) on the extraction yield.

As depicted in Fig. 6, 3-D response surface curves illustrated how the pairs of factors affected the extraction yield of alkaloids. All response surface curves were convex shape with a maximum point, indicating that the experimental ranges of factors were proper and reasonable. Accordingly, optimization conditions could be predicted by the response surface profile according to the fitted model. Form the profile of Fig. 6 (a), two factors strongly affected extraction yield, the predicted value at the vertex was 29.22 mg/g while at the optimal levels of phase ratio of 2.64 and particle size of 98.93 mesh. However, the predicted results obtained also had a little difference while at the optimal levels for each pair of factors. From Fig. 6 (b)–(f), the predicted value of the extraction yield were obtained from each 3-D curve at respective optimal levels while other variables kept at zero level. To sum up, predicted yields were in the range of 28.96–29.56 mg/g while the optimal levels were corresponding to the phase ratio of 2.61–2.64, the particle size of 96.55–99.48, the liquid-to-material ratio of 74.22–74.75 and extraction

temperature of 90.93–91.53°C, respectively. Thus, the process optimization was conducted to maximize the responses based on the "desirability" algorithm. The credibility of the optimum conditions was diagnosed through the desirability values ranged from 0 to 1. Then optimum conditions were determined while the combination of four factors yielded the highest desirability. Finally, the optimal combination of conditions concluded as the phase ratio of 2.62 for the ATPS, the liquid-to-material ratio of 74.53:1, the particle size of 96.15 mesh, extraction temperature of 90.88°C, and the extraction yield of alkaloids from RST was predicted to be 29.6268 mg/g.

3.3.3. Validation of optimum conditions

To validate applicability of the RSM model fitted, triplicate experiments were carried out to verify the optimum conditions. For convenient operation, extraction conditions were set at 2.60 of the phase ratio, 75:1 of the liquid-to-material ratio, 100 mesh of the particle size, 90°C of extraction temperature. The extraction yield of total alkaloids was 29.41±0.21mg/g through UV-vis analysis. The experimental results showed that the yield was very close to the prediction value, confirming that the model was adequate for the extraction process.

3.4. Comparison of other methods

In order to evaluate MAATE approach, a comparative study was conducted by using the conventional methods such as heating reflux extraction (HRE) and ultrasonic-assisted extraction (UAE). Using the ATPS of ethanol/Na_2HPO_4 system as extractant, all experiments were accomplished under following conditions: the phase ratio of 2.6, the particle size of 100 mesh, the liquid-to-material of 75:1 and extraction temperature of 90 °C besides extraction time as shown in Table 3. The obtained results were also listed in Table 3.

Table 3 The results of the extraction of alkaloids from *Radix Sophorae Tonkinensis* by different methods (n=3).

Method	Extraction solvent	Extraction time (min)	Total alkaloids (mg/g)	
			Top phase	Bottom phase
HRE	Ethanol/Na_2HPO_4/water	30	19.59±0.29	0.0208±0.0009
UAE	Ethanol/Na_2HPO_4/water	30	26.98±0.35	0.0473±0.0015
MAATPE	Ethanol/Na_2HPO_4/water	5	29.19±0.32	0.3820±0.0096

From the results in Table 3, almost all alkaloids were extracted from RST to the top phase by three methods, implying that the ATPE possessed advantages of selective extraction of target components. Moreover, MAATE demonstrated the strongest extraction capability compared to HRE and UAE. The extraction yield of alkaloids by MAATPE achieved up to 29.19±0.32 mg/g, which was far higher than that of HRE and UAE. The fact revealed that

extraction mechanism of MAATPE was different from other two methods. On the other hand, compounds in sample matrix may transfer selectively to two phases separated while demixing effect of the ATPS the spontaneously occurred. Relatively, single extraction solvent doesn't possess this function or ability, which is comparable to that the ATPS can do. In addition, the effect of microwave field on the ATPS and the sample matrix improved extraction efficiency. Thus, MAATPE is a potential and efficient alternative to extraction and purification of alkaloids from RST.

3.5. Identification of alkaloids by UPLC-Q-Orbitrap/MS

UPLC-Q-Orbitrap/MS is a high-resolution molecular recognition technique with a range of m/z 6000. Its accuracy and sensitivity are comparable with a triple quadrupole mass spectrometry (QQQ-MS), and the qualitative detection ability can reach 140000 FWHM (full width at half maximum), which is better than quadrupole-time of flight mass spectrometry (Q-TOF-MS) and Q-Trap mass spectrometry (Bastos et al., 2017; Bourmaud et al., 2016; Thermo. 2016; Zhang et al., 2017). Thus, identification of main alkaloids in the extract obtained by MAATPE was conducted by UPLC-Q-Orbitrap/MS for the composition characterization. With the help of XCalibur 3.0 software from ThermoFisher Scientific data processing system, mass fragmentation information for alkaloids was investigated by recording high solution data with. Full-MS-dd-MS^2 mode was used for detection of alkaloids, and certain formulas of RST were obtained by screening peaks in the chromatogram of total ion current within 3 ppm of quality error according to the nitrogen rule. The total ion chromatogram of alkaloids from RST was shown in Fig. 7, and the results of MS/MS detection were also listed in Table 4.

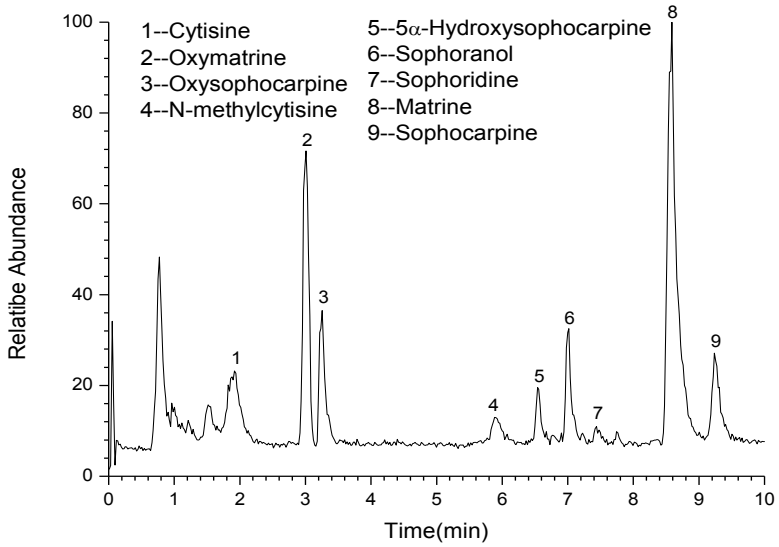


Fig. 7. Total ion chromatogram of alkaloids extracted from *Radix Sophorae Tonkinesis.*

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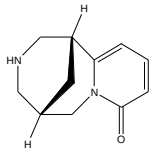
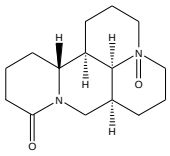
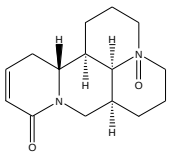
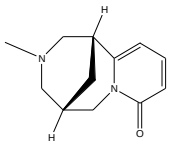
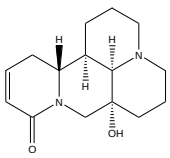
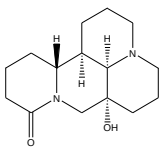
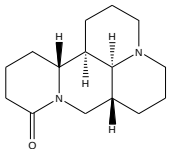
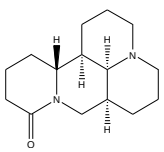
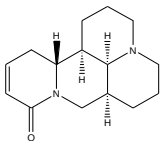
359 Table 4 The MS/MS data of alkaloids extracted from *Radix Sophorae Tonkinesis* by UPLC-Q-Orbitrap/MS.

No.	Retention time (min)	Analyte	Chemical structure	Theoretical $[M+H]^+$ (m/z)	Determined $[M+H]^+$ (m/z)	Product ions (m/z)	Error (ppm)	Collision energy (%)	Reference
1	1.95	Cytisine		191.11789	191.11774	162.09, 148.07, 123.09	-0.78	55	Przybył et al., 2007; Rouden et al., 2014; Zhao et al., 2011
2	3.06	Oxymatrine		265.19105	265.19067	247.18, 205.13, 176.10,150.12, 148.11,137.11, 136.11, 98.09, 96.08	-1.43	55	Chen et al., 2006; Pu et al., 1987; Zeng et al., 2015
3	3.25	Oxysophocarpine		263.17540	263.17499	245.16, 203.11, 177.13, 150.12, 148.11, 136.11, 110.09, 98.09, 96.08	-1.56	55	Ling et al., 2007; Zhao et al., 2011
4	5.94	N-methylcytisine		205.13354	205.13312	162.09, 146.05, 58.06	-2.05	50	Przybył et al., 2007; Zhao et al., 2011
5	6.56	5α-Hydroxysopho carpine		263.17540	263.17520	245.16, 195.14, 177.13, 150.12, 148.11, 122.09, 112.07, 110.06, 98.09, 96.04	-0.76	55	Saito et al., 1990
6	7.02	Sophoranol		265.19105	265.1908	247.18,188.14, 176.10, 150.12, 148.11, 122.09, 112.07, 98.06	-0.94	55	Zeng et al., 2015; Zhao et al., 2011
7	7.43	Sophoridine		249.19671	249.19635	176.10, 150.12, 148.11	-1.44	65	Pu et al., 1987; Zeng et al., 2015; Zhao et al., 2011
8	8.59	Matrine		249.19614	249.19580	176.10, 150.12, 148.11, 112.07, 98.09, 96.08	-1.36	65	Chen et al., 2006; Pu et al., 1987; Zeng et al., 2015; Zhao et al., 2011

9	9.25	Sophocarpine		247.18049	247.18011	245.16, 179.15, 150.12,148.11, 136.11, 96.08	-1.54	55	Pu et al., 1987; Zhao et al., 2011;

Considering the structural characteristics of alkaloids from RST, nine alkaloids in RST were identified and concluded in Table 4 by combining the MS/MS results of fragmentation information. Among those alkaloids, seven alkaloids were very similar in structure to tetracyclic quinolizidine. They had only one double-bond or hydroxyl group difference between each other and produced common product ions, showing that these product ions derived from the identical precursor ion (Chen et al., 2006; Pu et al., 1987). The characteristic product ions of m/z 150 and 148 indicated that these alkaloids were matrine-type. In addition, alkaloids with unsaturated ketone would produce characteristic product ions of m/z 245 or 177. According to main fragmentation pathway, seven alkaloids were respectively deduced as oxymatrine, oxysophocarpine, 5α-hydroxysophocarpine, sophoranol, sophoridine, matrine, sophocarpine based on experimental mass data and MS/MS references (Chen et al., 2006; Pu et al., 1987; Wang, et al., 2012a; Zeng et al., 2015; Zhao et al., 2011). The other two alkaloids were also identified as cytisine and N-methylcytisine (Chen et al., 2006; Han et al., 2016; Wang et al., 2012a; Zeng et al., 2015; Zhao et al., 2011). Furthermore, seven alkaloids were confirmed by comparing retention times and MS/MS data of the standards except for 5α-hydroxysophocarpine and sophoranol with similar researches reported.

3.6. Quantification of alkaloids by HPLC

3.6.1. HPLC separation

Quantification of alkaloids extracted from RST was performed by HPLC with UV detection. Nine alkaloids were completely separated on a C_{18} column at 30 °C in the gradient elution using the mobile phase consisting of methanol-acetonitrile-0.1% (w/v) ammonium solution. As shown in Fig. 8, nine alkaloids extracted from RST could be determined by HPLC due to suitability of the chromatographic system (their resolution, R>1.5 and number of theoretical plates, n>3000). Moreover, almost all alkaloids were extracted selectively from the sample matrix to the top phase whereas there were little left in the bottom phase. Thus, quantification of these alkaloids in RST would be characterized by the top-phase extract for analytical use.

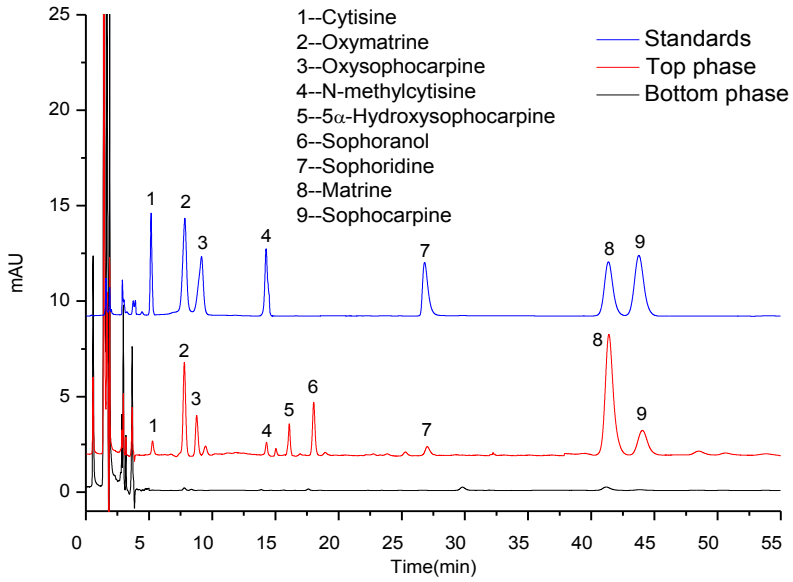


Fig. 8. HPLC chromatograms of mixed standard solution and samples extracted from *Radix Sophorae Tonkinensis*.

3.6.2. Linear range, limits of detection (LOD) and limits of quantification (LOQ)

In order to evaluate the performance of the HPLC method, the linearity of calibration curves for seven alkaloids was investigated by determining a series of mixed standard solutions at levels ranging from 1.00 to 200 μg/mL. Experimental data were fitted by linear regression according to the correlation between the peak area (A) and the concentration of the standards (C, μg/mL). Also, the limits of detection (LOD) and limits of quantification (LOQ) for the alkaloids were calculated by signal-to-noise ratio of 3:1 (S/N =3) and 10:1 (S/N =10). Finally, the results obtained were included in Table 5. Seven alkaloids had good linearity in the ranges of 2.00–200 μg/mL with correlation coefficients (R^2) higher than 0.9993. Their LODs and LOQs were in the range of 0.417–1.048 μg/mL and 1.351–3.396 μg/mL, respectively.

Table 5 The calibration curve, linear range, limits of detection and limits of quantification for alkaloids.

Alkaloids	Regression equation	R^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
Cytisine	A=15423C-13.01	0.9995	2.00~200	0.417	1.351
Oxymatrine	A=17643C+3.96	0.9994	2.00~200	0.526	1.704
Oxysophocarpine	A=13186C+7.50	0.9994	2.00~200	0.507	1.643
N-methylcytisine	A=15651C−9.01	0.9997	2.00~200	0.422	1.367
Sophoridine	A=15868C−10.28	0.9993	2.00~200	1.019	3.302
Matrine	A=27372C+6.82	0.9998	2.00~200	1.048	3.396

Sophocarpine	A=22432C+4.24	0.9999	2.00~200	1.040	3.370

3.6.3. Accuracy, precision and stability

To validate MAATPE-HPLC method, the recovery experiments were also accomplished by spiking samples at different concentrations for seven alkaloids. Precision and stability of the proposed method were also evaluated by determining alkaloids in the extracts according to MAATPE optimum conditions, and expressed by relative standard deviations (RSDs) of alkaloids. As shown in Table 6, the average recoveries of seven alkaloids were in the range of 85.18–106.3% with RSDs of 1.6–2.9%; RSDs of five samples tested fluctuated in the range of 0.9–2.1%. Furthermore, measurement RSDs at intervals of two hours were between 1.4% and 3.1%, indicating that alkaloids in the extract were stable within 12 hours. Thus, the proposed method could be applied to simultaneous determination of alkaloids in the herb sample.

Table 6 The results of recovery, precision and stability tests.

Alkaloids	Content (μg/mL)	Recovery test (n=3)				Precision (n=5)	Stability (12 h)
		Spiked (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (%)	RSD (%)	RSD (%)
Cytisine	14.71	15.03	30.69	106.3	2.9	1.2	2.6
Oxymatrine	104.6	105.3	210.6	100.7	1.7	1.4	1.5
Oxysophocarpine	53.62	50.10	105.1	102.7	2.3	1.7	1.8
N-methylcytisine	11.51	15.08	24.35	90.26	2.6	2.1	3.1
Sophoridine	16.47	15.08	31.12	97.14	2.5	1.5	2.3
Matrine	239.1	225.1	463.9	99.83	1.6	0.9	1.4
Sophocarpine	106.5	105.5	210.8	98.91	1.6	1.1	1.5

3.6.4. Quantification of alkaloids

In view of the above, MAATPE coupled HPLC-UV detection was applied to simultaneous extraction and determination of multiple alkaloids in RST for further investigation of their content distribution. According to MAATPE optimum conditions, alkaloids were extracted from the herb sample in triplicate, followed by HPLC analysis. From the results in Table 7, the contents of nine alkaloids demonstrated a significant difference, and ranked as follows: matrine>sophocarpine>oxymatrine>sophoranol>oxysophocarpine>5α-hydroxysophocarpine>sophoridine>cytisine>N-methylcytisine. It's remarkable that quinolizidine alkaloids like matrine-type were the dominant alkaloids in RST. Thus, RST is another resource of matrine-type alkaloids like *Sophora flavescens* Ait.

for pharmaceutical use (Han et al., 2016, Pan et al., 2015; Wang et al., 2012a; Xu et al., 2012; Yang at al., 2014; Zeng et al., 2015; Zhang, et al., 2015; Zhang, et al., 2016a).

Table 7 The results of determination of alkaloids in *Radix Sophorae Tonkinesis* (n=3).

Compound	Cytisine	Oxymatrine	Oxysophocarpine	N-methylcytisine	5α-hydroxysophocarpine	Sophoranol	Sophoridine	Matrine	Sophocarpine
Yield (mg/g)	0.632±0.009	4.476±0.050	2.304±0.037	0.493±0.008	1.423±0.028*	3.090±0.053*	0.708±0.015	10.284±0.083	4.578±0.042

***** Calculated by relative internal standard method.

3.7. Exploration of MAATPE mechanism

In the extraction process, MAATPE can not only significantly enhance alkaloids yield, but also selectively extract target compounds due to microwave field interaction and the ATPS formation property. In terms of the principles, the effect of microwave field depends strongly on the medium polarity, and the biphasic property of the ATPS affects greatly extraction capacity. For exploration of MAATPE mechanism, Fig. 9 illustrated the distribution of phase-forming components in the ATPS and the best results obtained by using water, ethanol, water-ethanol mixture and the ATPS under identical conditions. For the ATPS containing ethanol of 35.85% (w/w) and Na_2HPO_4 of 15.38% (w/w), among which ethanol of 86.03% and Na_2HPO_4 of 97.53% were distributed respectively to the top and bottom phases, introducing an extraction system compatible with alkaloids.

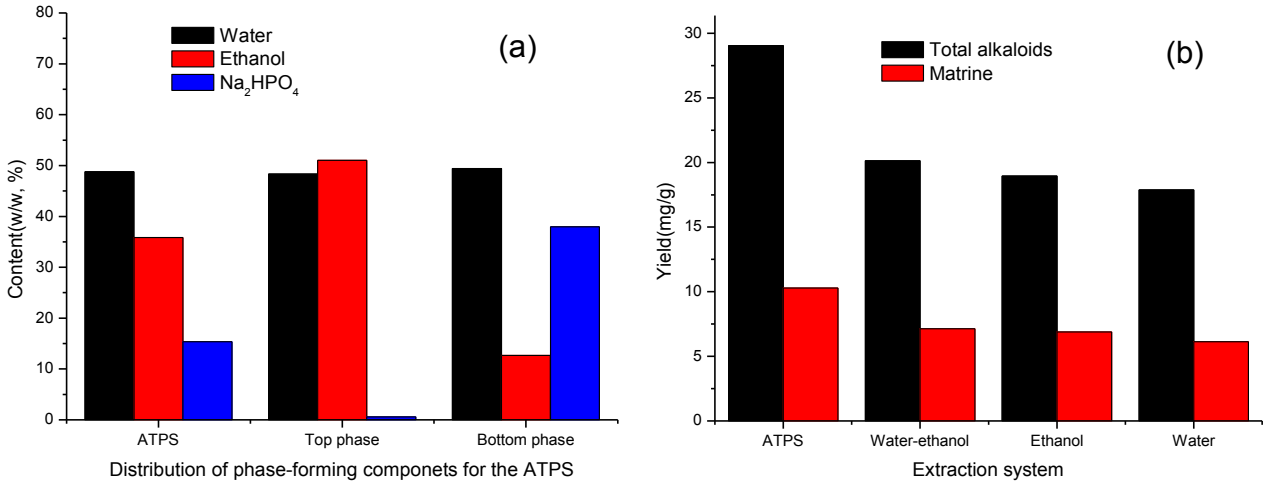


Fig. 9. The composition distribution of the ATPS for ethanol-Na_2HPO_4 system (a) and the effect of different extraction systems on the extraction yield of alkaloids (b) at 90 °C for 10 min using a closed-vessel microwave extraction device.

In general, microwave effect depends on the polarity of the extraction system that will determine how much intensity was produced by microwave filed. The higher the dielectric constant (or polarity) is, the stronger the thermal effect microwave interaction with it the medium produces. According to polarity difference, several

extraction systems were ranked in order of water>ethanol-water mixture>ATPS>ethanol except for the biphasic system of the ATPS. However, the results in Fig. 9 were not such case which sequence of the extraction yield was ATPS>ethanol-water>ethanol>water, and showed that stronger thermal effect didn't always bring the high yield. The fact revealed that the extraction of alkaloids was driven by not only thermal effect but also molecular miscibility. By comparison, the ATPS and the ethanol-water mixture demonstrated a great difference in extraction process even though containing same amounts of water and ethanol. It is particularly noteworthy that addition of Na_2HPO_4 would play a special role in the extraction process. In addition to increasing the medium polarity, the salt could impel ethanol-water mixture to be divided into two phase. As a result, extraction behaviors of alkaloids would be changed from the monophasic mode into the biphasic process, and thermal effect and demixing effect could be greatly enhanced with addition of the salt. Finally, alkaloids in RST were subjected to a series of multi-phase processes, migrating from sample matrix, the bottom salt-rich phase to the top ethanol-rich phase. Accordingly, the mass transfer of alkaloids among multi phases could be improved by MAATPE compared to a mono-phase solvent extraction. The reasons can be explained by the following three points. First, interaction of microwave field with medium molecules from whole extraction system was significantly intensified by ionic conduction and dipole rotation in presence of the inorganic salt. Second, coupling strength between molecules can be much enlarged, resulting in cell rupture and solute release (See Fig. 10 (a)). Third, multi-phase extraction equilibriums among sample matrix, top phase and bottom phase could be greatly accelerated by stronger thermal effect and demixing effect. Pictures of scanning electron microscopy (SEM) in Fig. 10 provided the evidence of the effect of the salt and medium on surface structure of the herb material under microwave field. More severe cell rupture exhibited that stronger impact of the ATPS and microwave field on the herb tissue than others, further confirming the above explanations of extraction process, higher yield and selectivity. It was also proved that is a powerful and prospective alternative to extract alkaloids from RST, carrying both characteristics from MAE and ATPE.

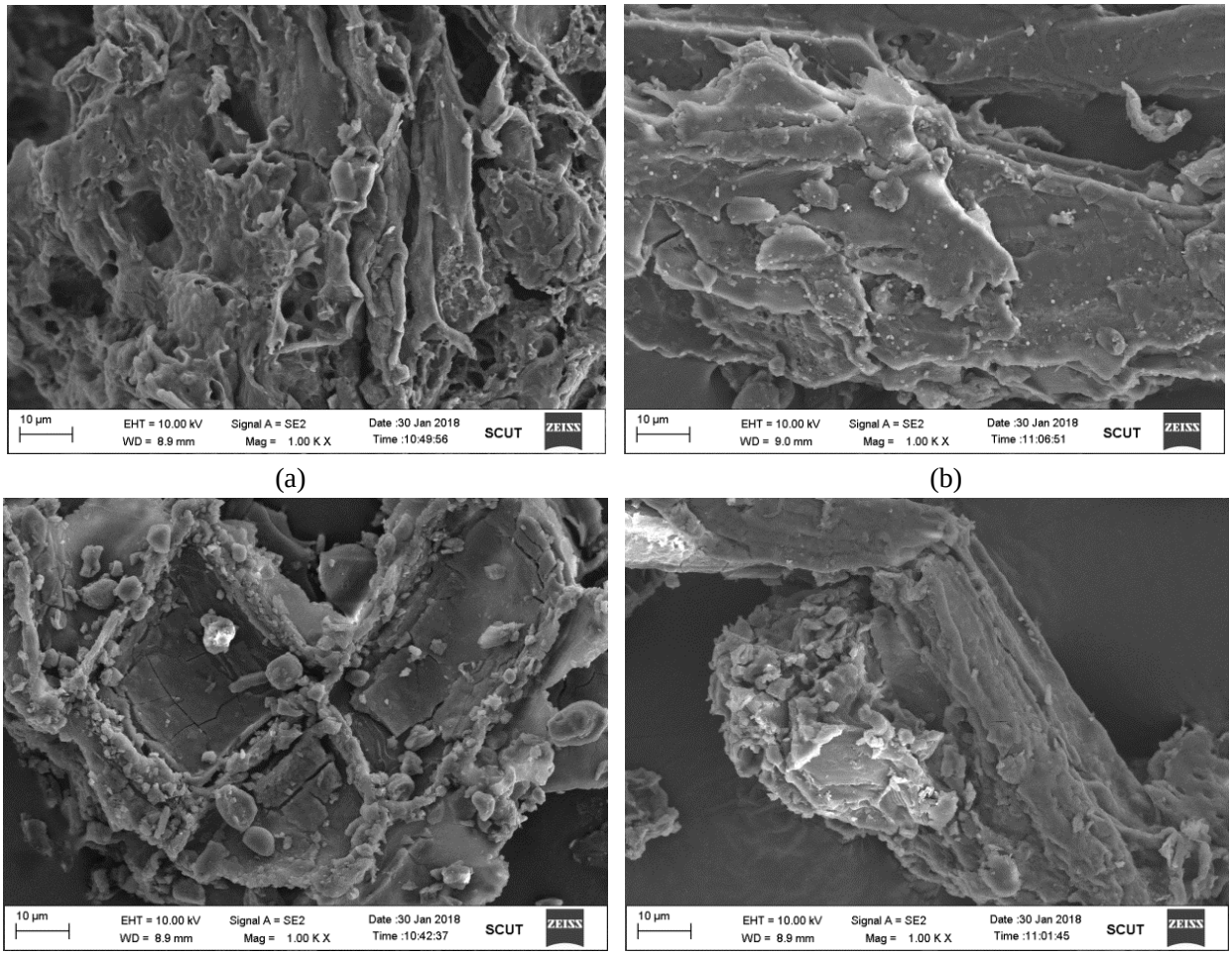


Fig. 10. The pictures of the herb material by scanning electron microscopy using the ATPS of ethanol-Na_2HPO_4 system (a), ethanol-water mixture (b), ethanol (c) and water (d) as extraction solvent.

4. Conclusion

In this study, a novel and rapid MAATPE method was introduced to extract alkaloids from *Rradix Sophorae Tonkinesis* (RST) using pressurized MAE system and the ATPS for ethanol-Na_2HPO_4 system. Key factors in the MAATPE process were investigated, followed by RSM optimization. Conditions optimized concluded as follows: the phase ratio of 2.60 for the ATPS, the particle size of 100 mesh, the liquid-to-material ratio of 75:1, extraction temperature of 90 °C and extraction time of 5 min. Identification and quantification of alkaloids extracted by MAATPE validated were accomplished by high-resolution UPLC-Q-Orbitrap/MS and HPLC-UV detection, respectively. According to the content from high to low, they were determined as matrine, sophocarpine, oxymatrine, oxysophocarpine, 5α-hydroxysophocarpine, sophoranol, cytisine, N-methylcytisine and sophoridine. Exploration of MAATPE mechanism demonstrated that the interaction of microwave field with the ATPS could not only improve the demixing effect and multi-phase mass transfer, but also cause a drastic change in the sample

matrix, especially in the presence of salt. Thus, MAATPE integrated MAE with ATPE into one-step procedure, exhibiting higher extraction efficiency and biphasic selectivity. It provided a green, efficient and promising alternative to extraction and separation of various alkaloids from natural products.

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