

# Desirability function approach for the optimization of microwave-assisted extraction of saikosaponins from *Radix Bupleuri*

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## Abstract

Microwave-assisted extraction (MAE) was applied to the extraction of saikosaponin a, c and d from *Radix Bupleuri*. Several operating parameters, namely microwave power, time, temperature and ethanol concentration, were optimized using response surface methodology (RSM) with a central composite rotatable design (CCRD). The ethanol concentration and time were found to be the most significant factors ( $p < 0.0001$ ) for the extraction of all three saikosaponins. By using the desirability function approach, the optimum MAE conditions to obtain desirable extraction yields for all these saikosaponins simultaneously were found at the microwave power of 360–400 W, ethanol concentration of 47–50%, temperature of 73–74 °C and time of 5.8–6.0 min. At these conditions, the yields from the verification experiments were 96.18–96.91% for saikosaponin a, 95.05–95.71% for saikosaponin c and 97.05–97.25% for saikosaponin d, which were in good agreement with the predicted values.

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## 1. Introduction

In recent years, interest in the research and development of herbal medicine has risen enormously, especially in the field of modernization of traditional Chinese medicines (TCM), which normally requires the extraction of effective components from herbal matrix. The dried root of *Bupleurum chinense* D.C., which is known as *Radix Bupleuri*, is one of the well-known TCM herbs and is used as a key ingredient for many Chinese multi-herb remedies, such as xiao-chai-hu-tang, a famous haematopoietic remedy in oriental medicine. It contains different forms of saikosaponins, such as saikosaponin a, c and d, which have been recognized as pharmacologically active compounds and possess immunomodulatory, hepatoprotective, anti-tumour and anti-viral activities [1,2]. The quality of *Radix Bupleuri* is generally determined by the contents of saikosaponins.

There were several studies focusing on the extraction, separation and analysis of saikosaponins from *Radix Bulerui* by

conventional methods [3–7]. The conventional methods for the extraction of effective components usually involves boiling the herbal matrices with water for 30–60 min to prepare the “herbal drinks” for oral intake, or soaking with ethanol or aqueous ethanol under moderate temperature for a relatively long time to leach the target compounds for being used in herbal prescription medicine or for analysis purpose. However, these conventional methods are very time-consuming and low efficient. Attention has therefore been drawn to the development of more efficient methods for rapid extraction, isolation and analysis of the effective components from medicinal plants.

Microwave-assisted extraction (MAE) has been successfully used in the extraction of effective compounds from different plant matrices in the past 5 years [8–18]. In our previous works, MAE was also employed for the extraction of effective components from various TCM herbs, including saikosaponins from *Radix Buplerui*. The results from these applications indicated that MAE can remarkably reduce the extraction time and solvent consumption, while offering better extraction efficiency. Our previous studies also showed that, when using the one-variable-at-a-time approach, the extraction yields of saikosaponin a, c and d were influenced by the levels of

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parameters such as the microwave power, irradiation time, temperature, ethanol concentration, solvent to sample ratio and sample particle size. More than 90% of the saikosaponins were extracted by using 2.00 g dried *Radix Bupleuri* with a particle size of 0.3–0.45 mm in 60 mL of 30–70% aqueous ethanol, 300–500 W of microwave power and at 75 °C for 3–5 min. However, no information is found about the interaction effects among the parameters on the extraction efficiency. Therefore, it is necessary to perform a systematic optimization to find the “best” conditions for MAE of saikosaponins from *Radix Bupleuri*.

Response surface methodology (RSM) with the desirability function approach has been proven to be a useful statistical tool to solve multi-variable problems and optimize one or several responses [19,20]. Moreover, personal computer, statistical software and computer graphics for desired function methodology implementation are now available and have been successfully applied in various industrial processes and researches to optimize conditions for sample preparation and analysis of analytes. Kwon et al. [15] used RSM to optimize the conditions for MAE of saponin components from ginseng roots; Lee et al. [21] optimized the extraction procedure for the quantification of Vitamin E using RSM; Carro and Lorenzo [22] used the desirability function to simultaneously optimize the solid-phase extraction of organochlorine and organophosphorus pesticides; Jimidar et al. [23] applied the desirability function to the selection of optimum separation conditions in capillary zone electrophoresis. Bourguignon and Massart [24] simultaneously optimized several chromatographic performance goals using the desirability function. The main advantage of using RSM is to reduce the experimental runs required to evaluate multiple parameters and their interactions. Therefore, it is less laborious and less time-consuming than other methods to optimize a process with one or more responses.

The objectives of this study are to better understand the relationships between the extraction yields of saikosaponins and the operating parameters such as microwave power, time, temperature and ethanol concentration, and to find a set of optimum conditions for MAE of saikosaponin a, c and d from *Radix Bupleuri* using RSM with the desirability function approach.

## 2. Materials and methods

### 2.1. Materials

*Radix Bupleuri* (dried root of *Bupleurum chinense* D.C.; Beichaihu) of 10.35% moisture content was obtained from Guangzhou Zhisheng Medicinal Company (Guangzhou, PR China). The *Radix Bupleuri* was grounded with a grinder and sieved to 0.30–0.45 mm, then packed in a polyethylene bag and stored at room temperature (23 °C) until used.

### 2.2. Reagents and apparatus

Chemicals used in this study included standards of saikosaponin a and c (Nacalai Tesque Inc., Tokyo, Japan) and

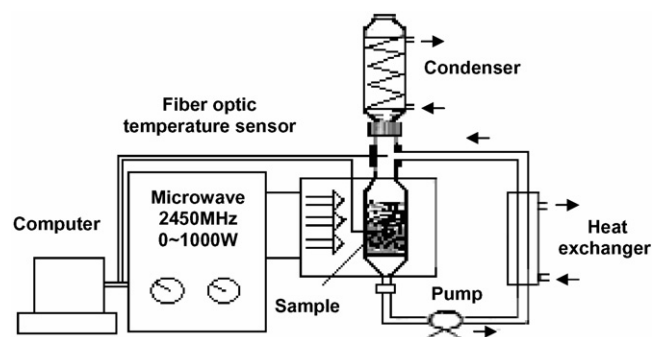


Fig. 1. Schematic diagram of microwave extractor.

saikosaponin d (Wako Pure Chemical Industries Ltd., Tokyo, Japan), methanol and acetonitrile (HPLC-grade, Tedia Company, Inc., USA), ethanol (analytical grade, Merck KgaA, Germany) and de-ionized distilled water.

All MAE experiments were carried out in a custom-made microwave reactor with cylindrical cavity (model 961, Microwave Power Consultants, VIC, Australia). This microwave reactor is a continuously variable microwave power system with power output up to 1000 W and a fibre optical temperature controller. The system is equipped with a glass extraction vessel topped by a condenser and works at atmospheric pressure. The sample was subjected to focused microwave irradiation to provide rapid heating and the solvent was circulated through the sample at a fixed flow rate of 4 mL s<sup>-1</sup> by a pump (Masterflex, IL, USA). A water bath (JULABO-F10, Julabo Labortechnik GmbH, Germany) was used for the cooling of the solvent in order to keep the microwave work with the setting power when the extraction temperature was achieved during extraction. The schematic diagram of the microwave extractor is shown in Fig. 1.

A centrifuge (Eppendor 5415D, Germany) was used for the separation of the extract from the matrix residue. A rotary evaporator (RE-52, Shanghai, China) was used for the concentration of extract.

A Hewlett Packard HP 1100 HPLC, equipped with a Quaternary pump (G1311A), a variable wavelength detector (G1314A) and a Rheodyne model 7255 manual injector with a fixed 20 μL sample loop, a Hypersil ODS C<sub>18</sub> (200 mm × 4.6 mm; 5 μm) reverse phase column (Hewlett Packard, USA) and a guard column (PN 96013, Alltech), were used for the analysis.

### 2.3. Experimental design

Based on our previous work, four operating factors, namely the microwave power ( $X_1$ ), irradiated time ( $X_2$ ), extraction temperature ( $X_3$ ) and ethanol concentration ( $X_4$ , % (v/v), ethanol in water) with five level settings were considered to be the independent variables, and the dependent variables were the extraction yields of saikosaponin a ( $Y_1$ ), saikosaponin c ( $Y_2$ ) and saikosaponin d ( $Y_3$ ). As shown in Table 1, total of 30 runs based on a central composite rotatable design (CCRD) with six center points were performed in random order with triplicates in each run.

Table 1  
Experimental conditions from the central composite rotatable design and experimental measurements of the responses

Run	Power, $X_1$ (W)	Time, $X_2$ (min)	Temperature, $X_3$ ( $^{\circ}$ C) <sup>a</sup>	Ethanol, $X_4$ (%)	Relative extraction yield (%) <sup>b</sup>		
					Saikosaponin a, $Y_1$	Saikosaponin c, $Y_2$	Saikosaponin d, $Y_3$
1	-1 (200)	-1 (3)	-1 (65)	-1 (35)	87.43 ± 1.68	81.79 ± 1.56	84.97 ± 0.46
2	-1 (200)	-1 (3)	-1 (65)	1 (65)	85.74 ± 1.51	81.26 ± 0.40	83.27 ± 1.04
3	-1 (200)	-1 (3)	1 (75)	-1 (35)	87.49 ± 2.21	84.41 ± 4.87	90.09 ± 2.31
4	-1 (200)	-1 (3)	1 (75)	1 (65)	84.91 ± 2.05	84.10 ± 3.08	85.70 ± 1.54
5	-1 (200)	1 (5)	-1 (65)	-1 (35)	91.16 ± 2.60	89.40 ± 4.56	92.82 ± 0.65
6	-1 (200)	1 (5)	-1 (65)	1 (65)	88.36 ± 1.18	90.94 ± 2.60	92.25 ± 0.93
7	-1 (200)	1 (5)	1 (75)	-1 (35)	92.58 ± 0.06	90.20 ± 0.76	93.39 ± 2.45
8	-1 (200)	1 (5)	1 (75)	1 (65)	88.08 ± 1.77	88.43 ± 4.49	91.25 ± 1.52
9	1 (400)	-1 (3)	-1 (65)	-1 (35)	87.30 ± 1.72	88.15 ± 2.97	86.21 ± 2.00
10	1 (400)	-1 (3)	-1 (65)	1 (65)	84.17 ± 0.74	86.61 ± 0.59	85.58 ± 0.11
11	1 (400)	-1 (3)	1 (75)	-1 (35)	90.49 ± 5.13	91.71 ± 1.69	91.08 ± 2.94
12	1 (400)	-1 (3)	1 (75)	1 (65)	87.35 ± 4.99	89.46 ± 0.71	89.31 ± 4.76
13	1 (400)	1 (5)	-1 (65)	-1 (35)	93.94 ± 0.79	90.83 ± 1.70	93.54 ± 1.38
14	1 (400)	1 (5)	-1 (65)	1 (65)	87.34 ± 2.16	90.30 ± 1.10	92.28 ± 1.49
15	1 (400)	1 (5)	1 (75)	-1 (35)	94.29 ± 4.09	92.33 ± 2.50	94.70 ± 2.25
16	1 (400)	1 (5)	1 (75)	1 (65)	93.25 ± 3.27	93.35 ± 1.71	92.82 ± 0.65
17	-2 (100)	0 (4)	0 (70)	0 (50)	90.20 ± 1.02	86.93 ± 2.59	90.32 ± 3.26
18	2 (500)	0 (4)	0 (70)	0 (50)	92.26 ± 1.77	92.48 ± 1.55	92.91 ± 2.39
19	0 (300)	-2 (2)	0 (70)	0 (50)	88.64 ± 1.35	83.49 ± 1.58	89.68 ± 0.22
20	0 (300)	2 (6)	0 (70)	0 (50)	94.23 ± 1.97	94.37 ± 1.23	95.43 ± 0.86
21	0 (300)	0 (4)	-2 (60)	0 (50)	88.95 ± 1.31	82.68 ± 0.37	85.65 ± 0.32
22	0 (300)	0 (4)	2 (80)	0 (50)	93.53 ± 1.65	93.33 ± 1.70	93.81 ± 0.57
23	0 (300)	0 (4)	0 (70)	-2 (20)	86.07 ± 4.05	74.08 ± 4.46	81.25 ± 0.95
24	0 (300)	0 (4)	0 (70)	2 (80)	84.72 ± 2.74	74.35 ± 0.28	80.52 ± 0.79
25	0 (300)	0 (4)	0 (70)	0 (50)	92.25 ± 1.09	91.34 ± 1.59	92.69 ± 1.56
26	0 (300)	0 (4)	0 (70)	0 (50)	94.02 ± 2.11	92.18 ± 2.98	93.29 ± 0.45
27	0 (300)	0 (4)	0 (70)	0 (50)	93.21 ± 2.00	92.24 ± 1.76	93.72 ± 0.82
28	0 (300)	0 (4)	0 (70)	0 (50)	93.78 ± 1.15	92.57 ± 0.83	94.40 ± 0.88
29	0 (300)	0 (4)	0 (70)	0 (50)	93.87 ± 1.98	94.91 ± 0.76	94.98 ± 0.87
30	0 (300)	0 (4)	0 (70)	0 (50)	94.39 ± 2.95	94.02 ± 0.96	93.95 ± 0.47

<sup>a</sup> Temperature can be controlled at  $\pm 0.5$   $^{\circ}$ C.

<sup>b</sup> Mean  $\pm$  S.D. ( $n=3$ ), relative yield of saikosaponin extracted by MAE to that by control extraction (conventional extraction for 8 h).

#### 2.4. Microwave-assisted extraction

Two grams of *Radix Bupleuri* samples were accurately weighed in the extraction vessel. The vessel was placed into the microwave cavity and 60 mL aqueous ethanol of different concentration was poured into the system. MAE with different levels of operating factors was carried out.

The extracts were then filtered and collected in a volumetric flask. The residue in the vessel was washed twice with  $\sim 30$  mL solvent and the washings were collected and combined with the extracts. The final volume of the combined filtrate was adjusted to 100 mL. Fifty millilitres of these extracts were evaporated under vacuum at 45  $^{\circ}$ C using a rotary evaporator. The evaporated residue was dissolved in 5 mL of HPLC-grade methanol, centrifuged at 200 rps for 10 min, and filtered through a PTFE syringe filter (0.45  $\mu$ m) for HPLC analysis.

#### 2.5. Conventional extraction

A conventional solvent extraction based on Park's work [7] was carried out as control for comparative purpose. Two grams of samples were extracted in a flask with 60 mL of 70%

ethanol at 45  $^{\circ}$ C for 8 h with agitation. The extraction matrix was re-extracted twice using fresh solvent. The extracts were then combined and prepared for HPLC analysis in the same procedure as above.

#### 2.6. HPLC analysis

The HPLC conditions were based on Kanazawa [4] and Park's work [7] with modifications. The gradient elution system consisted of acetonitrile (solvent I) and water (solvent II) and separation was achieved using the following gradient procedures: 0–10 min, 30% I, 70% II; 10–18 min, 30–40% I, 70–60% II; 18–28 min, 40–45% I, 60–55% II; 28–35 min, 45% I, 55% II; 35–40 min, 45–30% I, 55–70% II. The flow rate was fixed at 0.8 mL min<sup>-1</sup> and the absorbance was measured at a wavelength of 203 nm at room temperature. 10  $\mu$ L of the sample solution obtained from the extraction step was injected into the HPLC for analysis.

The analytes were quantified by comparing the chromatographic peak area with linear calibration at five concentration levels in the range from 0.05 to 1.0  $\mu$ g  $\mu$ L<sup>-1</sup> using saikosaponin a, c and d as external standards. The relative percentage extrac-

tion yield of each saikosaponin was defined as (mg saikosaponin obtained by MAE per g sample/mg saikosaponin obtained by conventional extraction per g sample) × 100%.

2.7. Statistical analysis and optimization

The mean values of the triplicate trials were fit to a second-order polynomial of the following form by the response surface regression procedure (RSREG) in the Statistical Analysis System (SAS 6.12):

$$Y_k = b_{k0} + \sum_{i=1}^4 b_{ki} X_i + \sum_{i=1}^4 b_{kii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 b_{kij} X_i X_j \quad (1)$$

where  $Y_k$  are the responses or dependent variables, with  $Y_1$  for the extraction yield of saikosaponin a,  $Y_2$  for saikosaponin c, and  $Y_3$  for saikosaponin d;  $b_{k0}$ ,  $b_{ki}$ ,  $b_{kii}$  and  $b_{kij}$  are the regression coefficients; and  $X_s$  are the coded independent variables (MAE operating factors). The fit of the model is evaluated by the  $R^2$  and the lack of fit.

In order to obtain a maximum yield for saikosaponin a, c and d simultaneously, the desirability function approach [20] was used. Derringer’s approach is first to convert each response,  $Y_k$ , into an individual desirability,  $d_k$ . The desirability scale ranges from 0 to 1, where if the response is outside an acceptable region, sets  $d_k = 0$ , and if the response is fully desirable (at its goal or target), sets  $d_k = 1$ . Considering the situation in the extraction of the saikosaponins by MAE, we wanted all yields to be as high as possible. Thus, a one-sided transformation was applied

$$d_k = \begin{cases} 0, & \text{if } Y_k \leq Y_k^{(\min)} \\ \left( \frac{Y_k - Y_k^{(\min)}}{Y_k^{(\max)} - Y_k^{(\min)}} \right)^r, & \text{if } Y_k^{(\min)} < Y_k < Y_k^{(\max)} \\ 1, & \text{if } Y_k \geq Y_k^{(\max)} \end{cases} \quad (2)$$

where  $Y_k^{(\min)}$  is the minimum acceptable value of  $Y_k$ ,  $Y_k^{(\max)}$  is the maximum value that is considered desirable and  $r$  is a positive constant. If  $r = 1$ , the  $d_k$  increases linearly as  $Y_k$  increases; if  $r > 1$ , the  $d_k$  changes more rapidly towards the  $Y_k^{(\max)}$ ; if  $r < 1$ , the  $d_k$  changes less rapidly towards the  $Y_k^{(\max)}$ .

The individual desirability functions from the considered responses are then combined to obtain the overall desirability  $D$ , defined as the geometric average of the individual desirability

$$D = (d_1, d_2, \dots, d_k)^{1/k} \quad (3)$$

where  $0 \leq D \leq 1$ , a high value of  $D$  shows that all  $d_k$ s are toward the target value, which is considered as the optimal solutions of the system.

2.8. Computer program and software

Statistical analysis and the search for optima were performed by a subroutine written in SAS (6.12). The Pareto chart and 3D plots of the responses were drawn using the software Statistica (6.0).

3. Results and discussion

3.1. Modeling the responses

The experimental data obtained from MAE given in Table 1 were analyzed by using the RSREG procedure in SAS (6.12).

The effects of independent variables on the relative percentage extraction yields ( $Y_1$ ,  $Y_2$  and  $Y_3$ , respectively) were tested for adequacy and fitness by the analysis of variance (ANOVA). As shown in Table 2, the  $R^2$  of each second-order polynomial regression was 0.9178, 0.9016 and 0.9374 for  $Y_1$ ,  $Y_2$  and  $Y_3$ , respectively, with no significant lack of fit at  $p > 0.05$ . The results indicated that the models used to fit the response variables were all significant ( $p < 0.01$ ) and adequate to repre-

Table 2  
Analysis of variance for the second-order polynomial models fitted to the responses  $Y_k$

Response	Source	Degrees of freedom	Sum of square	F-value	p-Value
$Y_1$ (saikosaponin a)	Model	14	304.801	11.968***	<0.0001
	Residual	15	27.288		
	Lack of fit	10	24.453	4.313	0.0602
	Pure error	5	2.835		
	$R^2$		0.9178		
$Y_2$ (saikosaponin c)	Model	14	785.851	9.813***	<0.0001
	Residual	15	85.806		
	Lack of fit	10	77.0181	4.382	0.0583
	Pure error	5	8.788		
	$R^2$		0.9016		
$Y_3$ (saikosaponin d)	Model	14	490.762	16.045***	<0.0001
	Residual	15	32.772		
	Lack of fit	10	29.507	4.519	0.0549
	Pure error	5	3.265		
	$R^2$		0.9374		

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

sent the relationship between the response and the independent variables.

The regression coefficients of the models for  $Y_1$ ,  $Y_2$  and  $Y_3$  are given in Table 3. The significance of each coefficient was determined by the  $p$ -value. The smaller the  $p$ -value is, the more significant is the corresponding coefficient. The results indicated that at the significant level of  $p < 0.05$ , the linear terms are all significant for all  $Y_k$ , except  $X_4$  for  $Y_2$ ; the quadratic terms are all significant for  $Y_1$ , but not significant for other responses except  $X_3^2$  for  $Y_3$  and  $X_4^2$  for  $Y_2$  and  $Y_3$ ; the cross-terms are not significant for all  $Y_k$ , except  $X_1X_3$  for  $Y_1$  and  $X_2X_3$  for  $Y_3$ .

### 3.2. Effect and mutual relationship of variables

The overall effects of each independent variable within the experimental range are given in Table 4. The results showed that  $Y_1$  is affected most significantly by ethanol concentration ( $X_4$ ) ( $p = 0.0001$ ), followed by time ( $X_2$ ) ( $p = 0.0001$ ), temperature ( $X_3$ ) ( $p = 0.01$ ) and power ( $X_1$ ) ( $p = 0.05$ ).  $Y_2$  is affected most significantly by ethanol concentration ( $X_4$ ) ( $p = 0.0001$ ), followed by time ( $X_2$ ) ( $p = 0.01$ ), power ( $X_1$ ) ( $p = 0.05$ ) and temperature ( $X_3$ ) ( $p = 0.1$ ).  $Y_3$  is affected most significantly by both ethanol concentration ( $X_4$ ) ( $p = 0.0001$ ) and time ( $X_2$ ) ( $p = 0.0001$ ), followed by temperature ( $X_3$ ) ( $p = 0.001$ ).

The linear, quadratic and cross-effects of each independent variable plotted in the form of Pareto chart are illustrated in Fig. 2, in which the bar lengths are proportional to the absolute values of the estimated effects and are used for comparing their relative importance. The effect is significant if its corresponding bar crosses the vertical line at the  $p = 0.05$  level.

It can be seen in Fig. 2 that the quadratic term of ethanol concentration ( $X_4^2$ ) gives the most important effect on all responses of  $Y_1$ ,  $Y_2$  and  $Y_3$ , followed by the linear term of time ( $X_2$ ). The third most important effect on  $Y_k$  is as  $X_4$  on  $Y_1$ ,  $X_1$  on  $Y_2$  and  $X_3$  on  $Y_3$ .

Fig. 2 also indicates other significant effects on  $Y_k$ . Fig. 2(a) shows the effects of the linear term of  $X_3$  and  $X_1$ , the quadratic term of  $X_1^2$ ,  $X_2^2$ ,  $X_3^2$ , and the cross-term of  $X_1X_3$  on  $Y_1$ . Fig. 2(b) shows the effects of the linear term of  $X_3$  on  $Y_2$ , and Fig. 2(c) shows the effects of the quadratic term of  $X_3^2$ , the cross-term of  $X_2X_3$  and both the linear terms of  $X_1$  and  $X_4$  on  $Y_3$ .

The effects of the independent variables and their mutual interaction on the extraction yields of  $Y_1$ ,  $Y_2$  and  $Y_3$  can also be visualized on the response surface and contour plots shown in Figs. 3–5.

As can be seen in Fig. 3,  $Y_1$ ,  $Y_2$  and  $Y_3$  increased significantly with increasing ethanol concentration from 20% to around 50%, then decreased significantly with increasing ethanol concentration from around 50% to 80% at any time range from 2.0 to 6.0 min (Fig. 3(a)), temperature from 60 to 80 °C (Fig. 3(b)) and power from 100 to 500 W (Fig. 3(c)). This indicates that ethanol concentration is one of the critical factors to be taken into consideration in MAE of saikosaponins, owing to its important implications on the solubility to the target compound, the

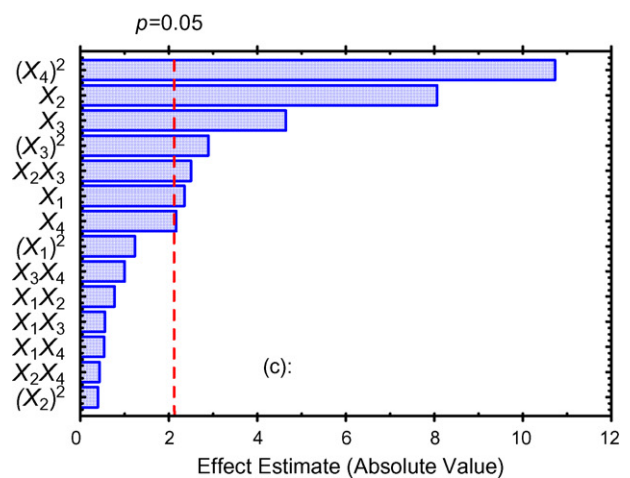
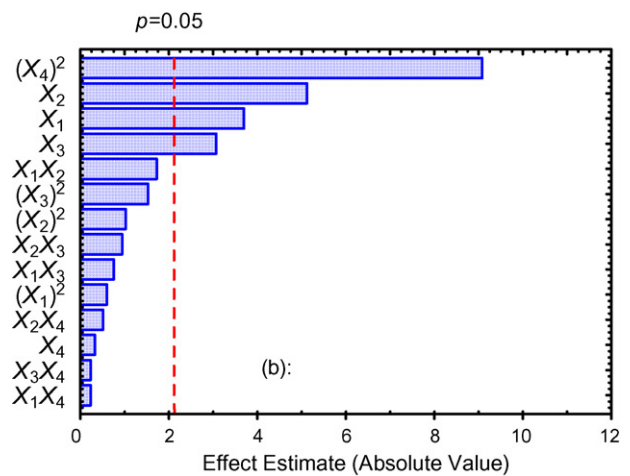
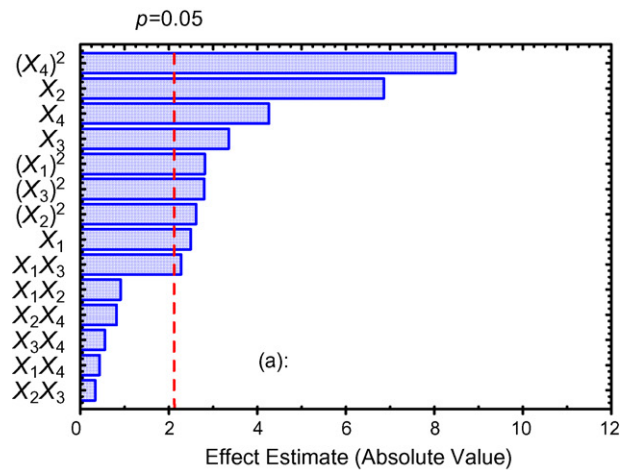


Fig. 2. Pareto chart of standardized effects for the relative extraction yield of saikosaponins. (a)  $Y_1$ , saikosaponin a; (b)  $Y_2$ , saikosaponin c and (c)  $Y_3$ , saikosaponin d.

interaction between the solvent and matrix, and the microwave absorbing properties.

The effect of time on  $Y_k$  can be seen in Figs. 3(a), 4(a) and (b), in which  $Y_1$ ,  $Y_2$  and  $Y_3$ , increased correspondingly with increasing time from 2.0 to 6.0 min for all levels of other variables.

Table 3  
Estimated coefficients from the fitted models for the responses  $Y_k$

Source	$Y_1$ (saikosaponin a)		$Y_2$ (saikosaponin c)		$Y_3$ (saikosaponin d)	
	Coefficient	$p$ -Value	Coefficient	$p$ -Value	Coefficient	$p$ -Value
$b_0$	93.5717		92.8767		93.8383	
$b_1$ ( $X_1$ : power)	0.6875*	0.0246	1.8046**	0.0022	0.7100*	0.0327
$b_2$ ( $X_2$ : time)	1.8875***	<0.0001	2.5021***	0.0001	2.4342***	<0.0001
$b_3$ ( $X_3$ : temperature)	0.9233**	0.0044	1.5004**	0.0077	1.4025***	0.0003
$b_4$ ( $X_4$ : ethanol)	-1.1742***	0.0007	-0.1596	0.7483	-0.6550*	0.0464
$b_{11}$	-0.7242*	0.0131	-0.2736	0.5580	-0.3471	0.2377
$b_{22}$	-0.6729*	0.0196	-0.4674	0.3223	-0.1121	0.6969
$b_{33}$	-0.7217*	0.0134	-0.6985	0.1469	-0.8183*	0.0110
$b_{44}$	-2.1829***	<0.0001	-4.1461***	<0.0001	-3.0296***	<0.0001
$b_{12}$	0.3062	0.3781	-1.0331	0.1045	-0.2875	0.4487
$b_{13}$	0.7662*	0.0382	0.4506	0.4627	0.2037	0.5895
$b_{14}$	-0.1462	0.6707	-0.1394	0.8186	0.1987	0.5986
$b_{23}$	0.1125	0.7433	-0.5644	0.3602	-0.9250*	0.0244
$b_{24}$	-0.2750	0.4275	0.3056	0.6167	0.1600	0.6712
$b_{34}$	0.1850	0.5913	-0.1406	0.8172	-0.3712	0.3310

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

The effect of temperature on  $Y_k$  can be seen in Fig. 3(b), in which  $Y_1$ ,  $Y_2$  and  $Y_3$  increased with increasing temperature from 60 to 70 °C for all ethanol concentration levels. No improvement in yields with a high level of temperature (>75 °C) was observed. Figs. 4(a) and 5 show that  $Y_1$ ,  $Y_2$  and  $Y_3$  increased with increasing temperature from 60 to 80 °C for levels of time from 2.0 to 6.0 min and power from 100 to 500 W, except that at time longer than 5.0 min and temperature higher than 75 °C, no increase in yield for saikosaponin d was observed. This might be explained by that the saikosaponin d was easily subjected to thermal degradation at a high temperature for a long time [25].

The effect of power is shown in Fig. 3(c). Increase in power from 100 to 500 W at any level of ethanol concentration led to an increase in  $Y_1$ ,  $Y_2$  and  $Y_3$ . The degree of the increase is similar to the effect caused by temperature, however, is less than the effect caused by ethanol concentration. Similar results were also observed in Fig. 4(b) at a high level of time from 4.0 to 6.0 min.

Fig. 5 shows that high levels of power and temperature together could lead to higher  $Y_1$ ,  $Y_2$  and  $Y_3$ .

### 3.3. Optimization

The objective of optimization was to find the MAE conditions which give the maximum extraction yields of each saikosaponin simultaneously. The desirability function approach was employed in the optimization procedure. For all responses,  $Y_k^{(\min)} = 93\%$  and  $Y_k^{(\max)} = 100\%$ , which were considered as the minimum and maximum values of the responses, were chosen for the optimization. According to Derringer's works [20], the value of the constant  $r$  is specified by the user. When  $r < 1$ , the desirability  $d_k$  first increases rapidly as  $Y_k$  starting to increase, then flattens as  $Y_k$  approach to  $Y_k^{(\max)}$ .  $r = 0.3$  was chosen as we found, in practice, 100% yield is difficult to be achieved; thus it would be desirable when the yield deviates moderately from  $Y_k^{(\min)}$ .

Table 4  
Analysis of variance for the overall effect of the independent variables on the response variables

Response	Independent variable	Degrees of freedom	Sum of square	$F$ -value	$p$ -Value
$Y_1$ (saikosaponin a)	$X_1$ : power (W)	5	36.965	4.064*	0.0156
	$X_2$ : time (min)	5	100.837	11.086***	0.0001
	$X_3$ : temperature (°C)	5	44.890	4.935**	0.0072
	$X_4$ : ethanol (%)	5	165.888	18.237***	<0.0001
$Y_2$ (saikosaponin c)	$X_1$ : power (W)	5	100.848	3.526*	0.0263
	$X_2$ : time (min)	5	179.910	6.290**	0.0024
	$X_3$ : temperature (°C)	5	76.080	2.660	0.0649
	$X_4$ : ethanol (%)	5	474.244	16.581***	<0.0001
$Y_3$ (saikosaponin d)	$X_1$ : power (W)	5	18.021	1.650	0.2074
	$X_2$ : time (min)	5	157.971	14.461***	<0.0001
	$X_3$ : temperature (°C)	5	82.136	7.519***	0.0010
	$X_4$ : ethanol (%)	5	265.293	24.285***	<0.0001

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

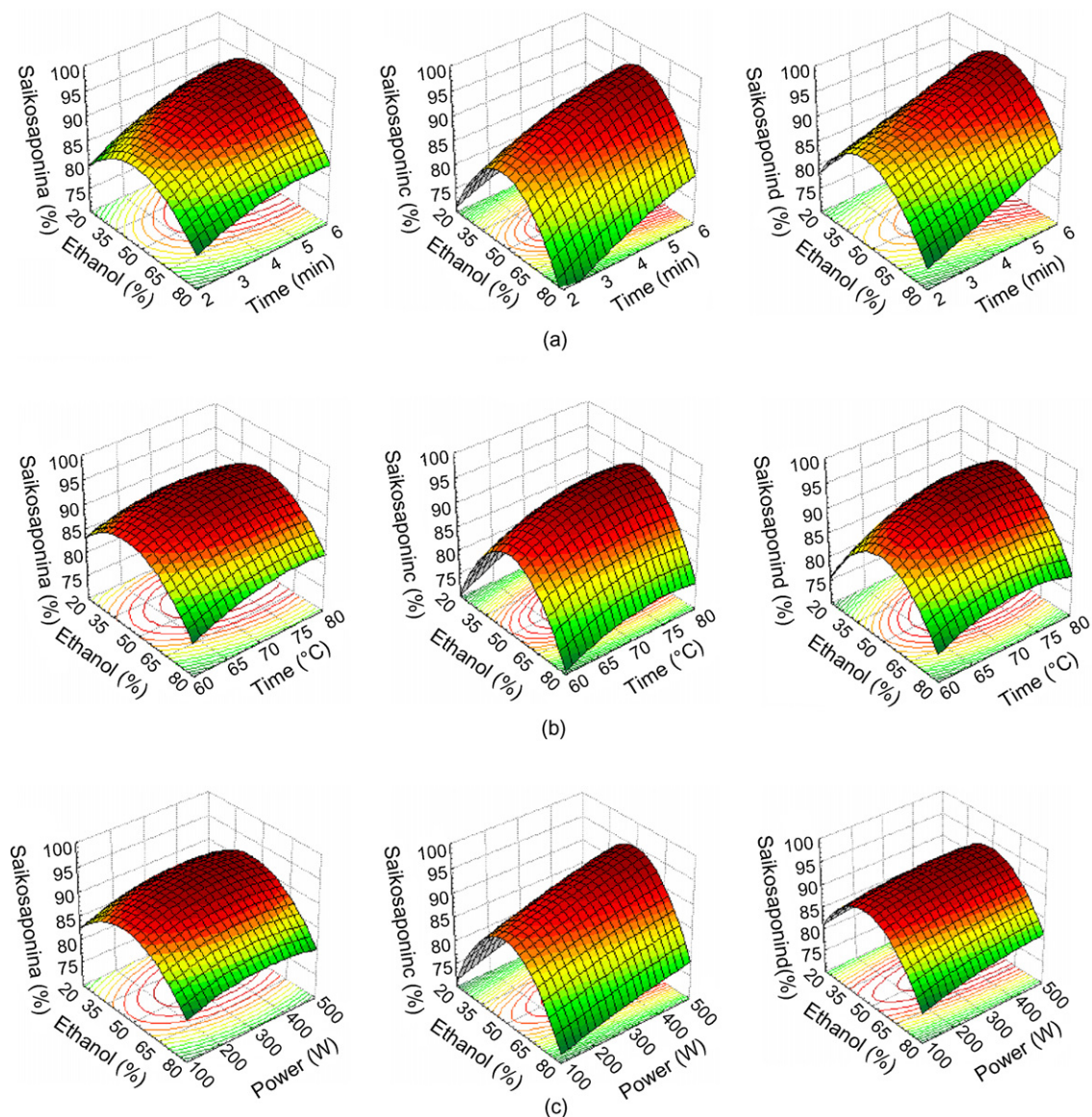


Fig. 3. Response surface and contour plots showing the effect of (a) ethanol concentration and time, (b) ethanol concentration and temperature and (c) ethanol concentration and power on yields of saikosaponin a, c and d. Other variables are constant at zero levels.

Table 5  
The predicted extraction yield of saikosaponins from optimization process

Case	<i>D</i> values	Optimized condition (coded level)				Predicted yield (%)		
		Power, $X_1$	Time, $X_2$	Temperature, $X_3$	Ethanol, $X_4$	Saikosaponin		
						a ( $Y_1$ )	c ( $Y_2$ )	d ( $Y_3$ )
1	0.805	0.6	2.0	0.6	-0.2	96.13	95.67	97.69
2	0.805	0.6	2.0	0.6	0.0	95.87	95.89	97.73
3	0.805	1.0	1.8	0.8	0.0	96.35	95.84	97.13
4	0.805	1.0	1.8	0.6	-0.2	96.43	95.65	97.33
5	0.806	1.0	1.8	0.6	0.0	96.17	95.85	97.37
6	0.806	0.8	2.0	0.6	-0.2	96.28	95.60	97.64
7	0.806	0.8	1.8	0.6	-0.2	96.34	95.70	97.40
8	0.806	0.8	2.0	0.6	0.0	96.02	95.82	97.68
9	0.806	0.8	1.8	0.6	0.0	96.10	95.91	97.44

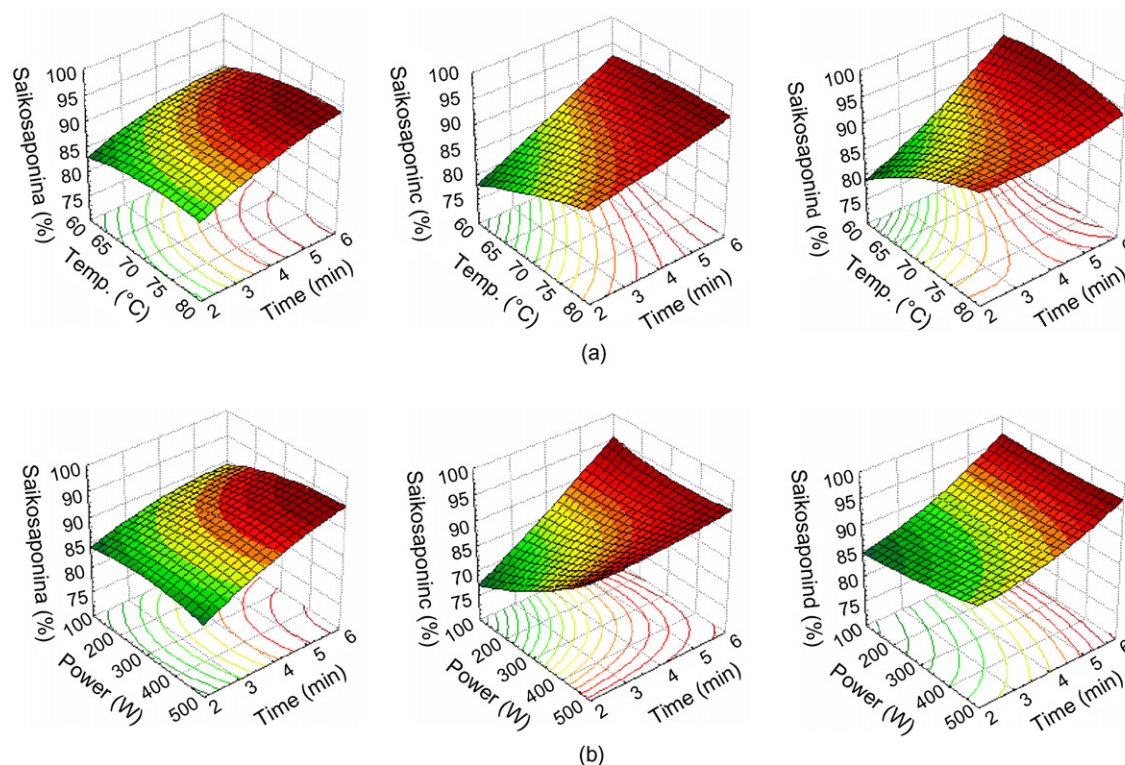


Fig. 4. Response surface and contour plots showing the effect of (a) time and temperature and (b) time and power on yields of saikosaponin a, c and d. Other variables are constant at zero levels.

For each independent variable, the coded level was taken from  $-2.0$  to  $2.0$  with a  $0.2$  interval. Using these values as constrains, a computer program was written first to calculate the individual desirability for each response by using Eq. (2), then to combine the individual desirability to obtain the overall desirability  $D$  by using Eq. (3), and finally to sort the maximum values of  $D$  and produce the optimum solutions for all responses. The nine results were obtained by running this program at the overall desirability  $D > 0.805$ , as shown in Table 5.

The results indicated that the coded levels of the optimized conditions were  $0.6$ – $1.0$  for power ( $360$ – $400$  W),  $1.8$ – $2.0$  for time ( $5.8$ – $6.0$  min),  $0.6$ – $0.8$  for temperature ( $73$ – $74$  °C) and  $-0.2$  to  $0.0$  for ethanol concentration ( $47$ – $50$ %), with the predicted yield of  $95.87$ – $96.43$ % for  $Y_1$ ,  $95.60$ – $95.91$ % for  $Y_2$  and  $97.13$ – $97.69$ % for  $Y_3$ .

### 3.4. Verification

In accordance with the optimization results obtained from RSM with the desirability function, verification experiments were carried out at the selected cases described in Table 6. The high, low and middle levels of each variable covered in the selected cases were taken into consideration.

The results indicated that the experimental values were  $96.18$ – $96.91$ % for  $Y_1$ ,  $95.05$ – $95.71$ % for  $Y_2$  and  $97.05$ – $97.25$ % for  $Y_3$  at the selected optimum conditions of  $360$ – $400$  W for power,  $5.8$ – $6.0$  min for time,  $73$ – $74$  °C for temperature and  $47$ – $50$ % for ethanol concentration. These experimental yields were in good agreement with the predicted values. Thus, it can be seen that the second-order models were adequate to describe the influence of the selected MAE

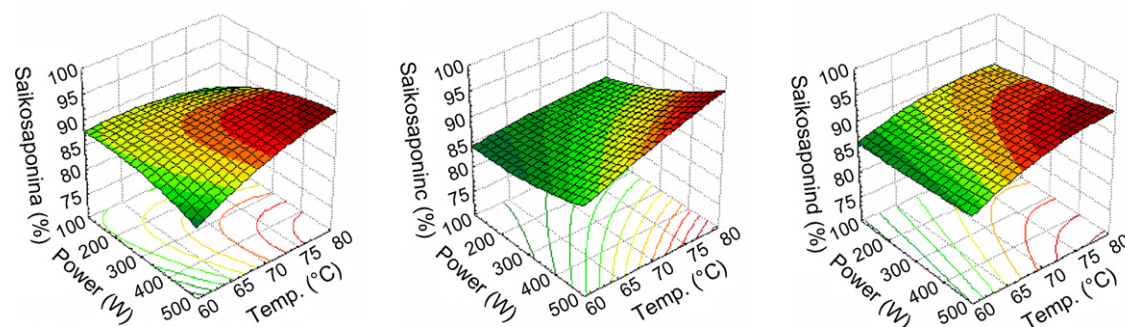


Fig. 5. Response surface and contour plots showing the effect of temperature and power on yields of saikosaponin a, c and d. Other variables are constant at zero levels.



Table 6  
The experimental extraction yield of saikosaponins at the selected optimized conditions

Case	Description of case		Optimized condition (true value)				Experimental yield (%) <sup>a</sup>		
	Level	Variable	Power, X <sub>1</sub> (W)	Time, X <sub>2</sub> (min)	Temperature, X <sub>3</sub> (°C)	Ethanol, X <sub>4</sub> (%)	Saikosaponin		
							a (Y <sub>1</sub> )	c (Y <sub>2</sub> )	d (Y <sub>3</sub> )
1	Low	X <sub>4</sub> , X <sub>3</sub> , X <sub>1</sub>	360	6.0	73	47	96.52 (±1.72)	95.71 (±1.12)	97.05 (±0.48)
	Middle	–							
	High	X <sub>2</sub>							
3	Low	X <sub>2</sub>	400	5.8	74	50	96.78 (±1.06)	95.05 (±0.97)	97.22 (±1.50)
	Middle	–							
	High	X <sub>4</sub> , X <sub>3</sub> , X <sub>1</sub>							
5	Low	X <sub>2</sub> , X <sub>3</sub>	400	5.8	73	50	96.18 (±1.27)	95.29 (±0.81)	97.22 (±1.13)
	Middle	–							
	High	X <sub>1</sub> , X <sub>4</sub>							
6	Low	X <sub>3</sub> , X <sub>4</sub>	380	6.0	73	47	96.91 (±0.88)	95.57 (±0.37)	97.25 (±1.48)
	Middle	X <sub>1</sub>							
	High	X <sub>2</sub>							
9	Low	X <sub>2</sub> , X <sub>3</sub>	380	5.8	73	50	96.61 (±1.01)	95.09 (±1.40)	97.16 (±1.08)
	Middle	X <sub>1</sub>							
	High	X <sub>4</sub>							

<sup>a</sup> Means from triplicate experiments (±S.D.).

operating variables on the relative extraction yields of saikosaponins.

#### 4. Conclusions

The saikosaponin a, c and d can be efficiently extracted using MAE. The variables of ethanol concentration and time showed a significant effect on the extraction yield for all three saikosaponins. The overall effect of temperature was more significant for saikosaponin d than for saikosaponin a, and was the least significant for saikosaponin c. The optimum conditions from the RSM with the desirability function approach were found to be 360–400 W of microwave power, 47–50% of ethanol concentration, 73–74 °C of temperature and 5.8–6.0 min of time, and were verified by experiments. At these conditions, the relative extraction yields of 96.18–96.91% for saikosaponin a, 95.05–95.71% for saikosaponin c and 97.05–97.25% for saikosaponin d were obtained simultaneously from the verification experiments. The experimental results were in good agreement with the predicted values.

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