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ORIGINAL PAPER

Enhanced ultrasound-assisted enzymatic hydrolysis extraction of quinolizidine alkaloids from *Sophora alopecuroides* L. seeds

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Abstract

Quinolizidine alkaloids are the main bioactive components in *Sophora alopecuroides* L. This study reports a novel ultrasound-assisted enzymatic hydrolysis method for the extraction of these important alkaloids. Box–Behnken design, a widely used response surface methodology, was used to investigate the effects of process variables on ultrasound bath-assisted enzymatic hydrolysis (UAEH) extraction. Four independent variables, pH, extraction temperature (°C), extraction time (min) and solvent-to-material ratio (mL/g), were studied. For the extraction of sophocarpine, oxysophocarpine, oxymatrine, matrine, sophoramine, sophoridine and cytisine, the optimal UAEH condition was found to be a pH of 5, extraction temperature of 54 °C, extraction time of 60 min and solvent-to-material ratio of 112 mL/g. The experimental values obtained under optimal conditions were fairly consistent with the predicted values. UAEH extraction was then compared with reflux heating, enzymatic extraction and ultrasound-assisted extraction. Of these extraction methods, UAEH extraction under optimal conditions produced the highest yield for seven types of alkaloids. In addition, UAEH extraction resulted in lower ingredient degradation than reflux heating extraction.

Keywords Ultrasound bath-assisted enzymatic hydrolysis · Quinolizidine alkaloids · Response surface methodology · *Sophora alopecuroides* L. · HILIC–UHPLC–TQ-MS/MS

Introduction

Quinolizidine alkaloids (QAS) from *Sophora alopecuroides* L. have been reported to have a large range of bioactive properties, including neuroprotective [1], analgesic [2], antioxidant [3], cardioprotective [4], hypothermic and antipyretic [5], anti-tumor [6, 7] and anti-hepatitis B [8] properties. In addition to their use as pharmaceuticals, these alkaloids are used as pesticides with no residue issues [9]. While QAS are contained in many different parts of *S. alopecuroide*s, the QAS content is highest in the seeds [10]. Currently, *S. alopecuroides* seeds are the best sources for QAS extraction. Industrially, QAS are extracted using a boiling method. However, some QAS, such as oxymatrine and oxysophocarpine, are degraded by high-temperature extraction [11], and so milder extraction conditions are desirable.

Advanced extraction methods, such as supercritical fluid extraction (SFE) [12], microwave-assisted extraction (MAE) [13, 14], enzymatic extraction (EE) [15] and ultrasonic-assisted extraction (UAE) [16], have been developed and used to extract compounds from plant sources. However, all of these methods have drawbacks. For example, most

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conventional procedures and enzymatic extractions are time-consuming and suffer from low extraction efficiencies. SFE requires the use of complex and expensive equipment, and MAE does not allow for the extraction of thermolabile analytes. Therefore, a QAS extraction method that can be operated under economical, environmentally friendly and controllable conditions is desirable.

Enzymatic extraction (EE) is a commonly used method for the extraction of compounds from plant sources [15]. Enzymatic extraction processes are carried out under mild conditions and can minimize the loss of thermolabile compounds. Moreover, because seeds contain large amounts of polysaccharides [17] that can hinder solvent penetration during extraction, enzymatic hydrolysis by cellulase can facilitate the release of intracellular chemical constituents [18]. The main drawback of enzymatic hydrolysis is the long time required to complete the process [19]. Thus, this study seeks to speed up the enzymatic extraction procedure while guaranteeing the quantitative release of the desired compounds.

Recently, ultrasound energy has been used to facilitate the enzymatic extraction of biological materials [20, 21]. When ultrasound waves are applied, an induced cavitation process occurs in the liquid, promoting an increase in pressure and temperature via bubble collapse. When a solid phase is immersed in the liquid phase, the asymmetric cavity collapses, producing high-speed liquid jets that impact the solid surface. This process results in high analyte transport rates from the solid particles to the liquid phase (extracting solution). Such high transport rates usually shorten the extraction or pretreatment time. Ultrasound baths are ideal when strict temperature control is needed (e.g., enzymatic hydrolysis).

Given the properties of ultrasound discussed above, exploring the simultaneous use of ultrasonication and biocatalysis for QAS extraction is an interesting undertaking. In this study, we investigate the effectiveness of ultrasound bath-assisted enzymatic hydrolysis for QAS extraction from *S. alopecuroides* seeds. The study includes (a) the optimization of ultrasound bath-assisted enzymatic hydrolysis (UAEH) parameters (pH, extraction temperature, extraction time and solvent-to-material ratio) for a combination of seven QAS using a response surface methodology and (b) a comparative assessment of the yields obtained by UAEH, conventional, enzymatic and ultrasound-assisted extraction for seven QAS.

Materials and methods

Plant materials

S. alopecuroides seeds were collected from Yanchi (Ningxia, China) in September 2013. The botanical origin of the seeds was identified by one of the authors (H.W.), and voucher specimens were deposited at the Herbarium in Ningxia Medical University, P. R. China. The materials were pulverized to homogeneous powders (40 mesh).

Chemicals and reagents

Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany), and deionized water (H_2O) was purified using a Milli-Q water purification system (Millipore, Billerica, MA, USA). Other reagent solutions, such as ammonium acetate, acetic acid and cellulase (15000 U/g), were of analytical grade (Sino Pharm Chemical Reagent Co., Ltd., Shanghai, China). Chemical standards of matrine, oxymatrine, sophocarpine, oxysophocarpine, sophoridine, sophoramine and cytisine were purchased from Chengdu Must Co. (Chengdu, China). The structures of these compounds are shown in Fig. 1. The purity of each reference compound was determined to be over 98% by UHPLC/ESI-MS.

The amount of enzyme for extraction

In a preliminary study, different amounts (0.5, 1, 2, 4 and 6% w/w) of cellulase were used for the UAEH extraction of *S. alopecuroides* seeds. UAEH was performed under the following conditions: pH 5, 50 °C, solvent-to-solid ratio of 100 mL/g and a time of 45 min. The amount of cellulase that resulted in the highest yield of seven alkaloids was used in further experiments to optimize alkaloid extraction from *S. alopecuroides* seeds.

Ultrasound bath-assisted enzymatic hydrolysis

UAEH extractions were carried out with an ultrasonic device (KH-500DV, Kunshan Ultrasonic Instrument Co. Jiangsu, China) equipped with a digital timer and a temperature controller. Dried powdered seeds (0.25 g) were weighed into a 50-mL conical flask containing the previously determined optimal amount of enzyme. Samples were then sonicated for various times (20, 45, 70 min) at various temperatures (40, 50, 60 °C), pHs (4, 5, 6) and solvent-to-material ratios (60, 100, 140 mL/g). After extraction, solvent was added to compensate for the weight lost during extraction. The solution was mixed thoroughly, then centrifuged at 13,000 rpm for 10 min. All of the solutions were then filtered through a 0.22-μm membrane filter before being injected into a UHPLC–MS/MS system for analysis. The effect of the extraction time, temperature, pH and solvent-to-material ratio were then assessed.

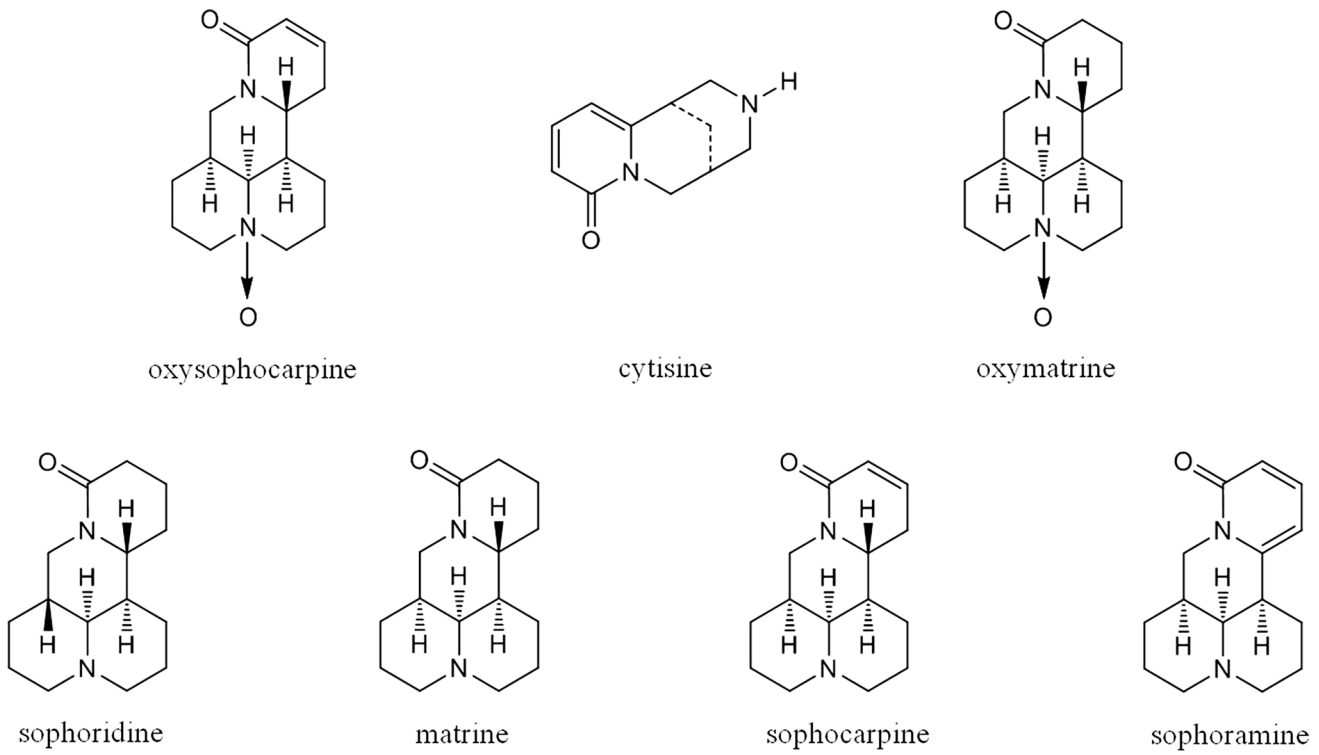

Fig. 1 Chemical structures of the seven alkaloids

Table 1 Precursor/product ion pairs and parameters for the MRM of the compounds used in this study

Analyte	Retention time (min)	$[M + H]^+$ (*m/z*)	MRM transitions (precursor → product)	Cone voltage (V)	Collision energy (eV)
1 Sophocarpine	3.52	247	247 → 136	46	30
2 Oxysophocarpine	3.59	263	263 → 245	42	28
3 Oxymatrine	4.14	265	265 → 205	44	28
4 Matrine	4.18	249	249 → 148	38	30
5 Sophoramine	4.3	245	245 → 122	46	32
6 Sophoridine	4.59	249	249 → 176	20	36
7 Cytisine	4.62	191	191 → 148	34	20

Conventional extraction, enzymatic extraction and ultrasound-assisted extraction

Industrially, alkaloids from *S. alopecuroides* seeds are extracted by boiling for various times with water as the solvent. We used the reflux heating (RH) method in the laboratory with only slight modification; the incubation time was 2 h and the temperature was 90 °C. The conditions used for enzymatic extraction (EE) were the same as those used in the optimized UAEH method with the exception of the extraction time. In this study, longer incubation times (2, 4, 6, 8 and 10 h) were investigated due to the low efficiency of enzymatic hydrolysis. The conditions used for ultrasound-assisted extraction (UAE) were consistent with those used in the optimized UAEH method, except that an enzyme was not used in UAE method.

Table 2 Variable levels used in the experimental design

Symbols	Independent variables	− 1	0	+ 1
X_1	pH	4	5	6
X_2	Extraction temperature (°C)	40	50	60
X_3	Extraction time (min)	20	45	70
X_4	Solvent-to-material ratio (mL/g)	60	100	140

Table 3 Box–Behnken design (uncoded) arrangement for extraction and the responses of contents of the alkaloid compounds (μg/g)

Run	X_1	X_2 (°C)	X_3 (min)	X_4 (mL/g)	Contents of analytes (mean ± SD, $n = 3$)						
					Sophocarpine	Oxysophocarpine	Oxymatrine	Matrine	Sophoramine	Sophoridine	Cytisine
1	5	50	20	140	377.05 ± 9.86	13519.52 ± 569.18	13708.52 ± 523.41	376.17 ± 13.86	282.15 ± 9.29	8543.69 ± 334.21	8742.98 ± 299.43
2	4	50	20	100	343.08 ± 8.24	12030.26 ± 424.05	11696.08 ± 359.82	345.76 ± 13.52	239.12 ± 7.20	7709.72 ± 307.57	7429.02 ± 270.01
3	4	50	70	100	365.41 ± 9.12	12935.53 ± 352.72	12970.53 ± 399.02	367.92 ± 14.38	268.59 ± 7.91	8280.12 ± 362.66	8652.19 ± 309.82
4	4	50	45	140	350.32 ± 8.39	12407.36 ± 431.25	12002.42 ± 375.07	352.83 ± 13.79	252.65 ± 8.58	7843.76 ± 313.56	8249.26 ± 268.76
5	5	40	70	100	363.12 ± 5.99	12652.43 ± 340.83	12652.43 ± 390.22	380.08 ± 14.43	279.92 ± 9.48	8022.94 ± 361.49	8679.27 ± 323.79
6	6	50	45	140	367.18 ± 9.11	12891.43 ± 404.23	12856.83 ± 402.56	370.84 ± 13.98	275.86 ± 9.00	8078.39 ± 353.83	8636.83 ± 302.28
7	4	60	45	100	347.18 ± 8.33	12073.82 ± 422.58	11586.88 ± 361.19	348.93 ± 13.79	249.87 ± 8.57	7724.85 ± 318.34	8026.35 ± 248.93
8	4	50	45	60	343.72 ± 6.12	12057.36 ± 322.76	11801.08 ± 266.83	343.12 ± 11.93	237.63 ± 7.26	7632.17 ± 324.28	7628.36 ± 222.26
9	4	40	45	100	323.59 ± 7.42	11642.33 ± 409.48	10632.24 ± 333.59	364.83 ± 15.25	235.97 ± 7.75	7684.54 ± 310.52	7382.52 ± 258.38
10	5	60	45	60	385.12 ± 8.85	14427.58 ± 504.96	13531.58 ± 508.19	389.84 ± 14.90	283.60 ± 9.21	8680.43 ± 395.20	8810.30 ± 328.36
11	5	60	45	140	395.64 ± 9.08	14600.52 ± 515.01	13988.52 ± 433.64	398.74 ± 15.98	294.12 ± 9.74	8978.43 ± 393.26	8951.25 ± 323.29
12	6	50	45	60	347.01 ± 8.38	12178.82 ± 429.25	11881.58 ± 328.09	366.36 ± 14.39	224.37 ± 7.39	7535.67 ± 310.26	7992.92 ± 257.22
13	5	40	20	100	358.79 ± 8.39	12477.36 ± 437.70	12366.94 ± 385.37	361.09 ± 14.76	257.93 ± 8.46	7935.67 ± 367.58	8557.83 ± 299.43
14	5	50	70	60	395.42 ± 9.12	14765.43 ± 519.79	13651.47 ± 432.19	405.76 ± 15.91	281.91 ± 9.68	8637.61 ± 410.32	8976.41 ± 324.17
15	6	40	45	100	378.01 ± 8.87	13524.56 ± 565.25	13419.16 ± 415.99	355.84 ± 13.99	252.74 ± 8.09	7880.67 ± 345.25	8798.12 ± 300.93
16	5	40	45	140	365.44 ± 9.82	12954.21 ± 453.39	12954.74 ± 408.78	365.82 ± 14.89	264.31 ± 8.38	8285.98 ± 382.81	8678.53 ± 313.74
17	5	50	45	100	413.23 ± 9.65	15258.25 ± 538.03	14200.44 ± 450.21	405.02 ± 15.79	306.17 ± 10.24	9727.66 ± 426.18	9025.23 ± 345.88
18	6	50	20	100	364.19 ± 8.51	13284.88 ± 461.97	13355.62 ± 424.02	333.63 ± 12.99	262.23 ± 8.44	8111.41 ± 315.17	8625.25 ± 301.39
19	5	50	45	100	404.77 ± 9.27	14995.10 ± 534.82	14092.72 ± 446.81	413.03 ± 16.20	300.91 ± 9.95	9480.39 ± 399.08	9176.41 ± 341.17
20	5	50	45	100	417.58 ± 11.01	14840.33 ± 520.41	14740.81 ± 556.96	414.53 ± 16.18	306.50 ± 9.92	9933.45 ± 465.91	9325.35 ± 350.38
21	5	50	45	100	403.82 ± 9.83	14458.29 ± 506.04	14004.06 ± 534.59	416.81 ± 16.25	299.47 ± 9.27	9745.04 ± 455.73	9200.12 ± 322.45
22	6	60	45	100	385.19 ± 8.18	14165.47 ± 486.94	13628.66 ± 522.48	385.03 ± 15.09	284.78 ± 9.34	9074.39 ± 367.45	8833.54 ± 329.17
23	5	60	20	100	376.67 ± 9.92	13490.68 ± 472.17	13300.58 ± 528.93	378.83 ± 14.25	277.44 ± 9.22	8280.52 ± 372.28	8699.53 ± 304.48
24	5	50	45	100	408.18 ± 9.75	14987.45 ± 524.56	14055.62 ± 536.78	418.15 ± 16.34	311.27 ± 10.12	9678.43 ± 443.91	9264.52 ± 349.25
25	5	50	70	140	418.71 ± 10.87	14165.47 ± 495.78	14263.32 ± 442.38	410.93 ± 15.99	320.52 ± 10.51	9853.72 ± 431.59	9205.09 ± 322.17
26	5	50	20	60	380.91 ± 8.92	13680.66 ± 488.82	13371.47 ± 414.51	372.27 ± 14.59	278.92 ± 9.27	8327.39 ± 334.73	8733.54 ± 301.67
27	5	60	70	100	397.13 ± 9.13	14667.34 ± 612.35	13699.16 ± 424.62	396.49 ± 15.31	305.51 ± 9.99	9623.97 ± 431.58	8980.11 ± 334.57
28	6	50	70	100	372.38 ± 7.59	13311.29 ± 463.12	13628.66 ± 422.48	368.39 ± 14.79	275.58 ± 8.92	8110.37 ± 355.33	8712.75 ± 354.63
29	5	40	45	60	358.59 ± 7.13	12590.26 ± 341.76	12118.44 ± 386.73	363.84 ± 14.36	260.92 ± 8.59	7935.67 ± 313.47	8512.29 ± 298.76

UHPLC–MS analysis of extracts

UHPLC was performed using a Waters ACQUITY UHPLC system (Waters, Milford, MA, USA). Hydrophilic interaction chromatographic separation was performed on an ACQUITY UHPLC BEH Amide column (2.1 × 100 mm, 1.7 µm). The mobile phase was composed of A (0.1% formic acid and 10 mM ammonium acetate in aqueous solution) and B (0.1% formic acid in acetonitrile) with a gradient elution: 0–3 min, 10–13% A; 3–4 min, 13% A; 4–5 min: 13–20% A. The mobile phase flow rate was kept constant at 0.4 mL min^{-1}, and the column temperature was maintained at 35 °C with a column temperature oven. To remain within the linear range of the standard curve, filtrates were diluted 100-fold. A 1-µL aliquot was injected into the UHPLC system for analysis. The column eluent was directed to the mass spectrometer. All analyses were conducted using MassLynx™ XS software.

Mass spectrometry detection was performed using a Waters Xevo TQ tandem quadrupole mass spectrometer (Micromass MS Technologies, Manchester, UK) with an ESI source operated in the positive ion mode. The source parameters were set as follows: capillary voltage, 3.0 kV; desolvation gas flow, 1000 L h^{-1}; desolvation temperature, 550 °C; cone gas flow, 50 L h^{-1}; source temperature, 150 °C. Data were collected in the multiple reaction monitoring (MRM) mode by simultaneously screening parent and daughter ions. Cone voltage (CV) and collision energy (CE) were optimized individually for each target compound. The parameters selected for each compound are given in Table 1. The dwell time was automatically set by MassLynx.

Experiment design

A response surface methodology (RSM) was used to investigate the influence of four independent variables on the extraction efficiency of matrine, oxymatrine, sophocarpine, oxysophocarpine, sophoridine, sophoramine and cytisine. The pH (X_1), extraction temperature (°C, X_2), extraction time (min, X_3) and solvent-to-material ratio (mL/g, X_4) were chosen as the independent variables to be optimized. Experiments were performed based on a Box–Behnken design (BBD). Table 2 shows the ranges and center point values of the four independent variables. The complete design was carried out in random order and consisted of 29 treatments, including five replicates at the central point (Table 3). Data were analyzed by multiple regression to fit the following quadratic polynomial model:

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

where Y is the predicted response, β_0 is a constant, and β_i, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients of the model, respectively. X_i and X_j represent independent variable levels.

Statistical analysis

The experimental results obtained from the response surface design were analyzed with Design-Expert 8.5 software (Trial version, State-Ease Inc., Minneapolis, MN, USA). p values less than 0.05 were considered to be statistically significant. The experimental results were expressed as the mean ± SD. All analyses were performed in triplicate.

Results and discussion

Effect of the enzyme amount on the alkaloid yield

In the present study, QAS were represented by seven major compounds: sophocarpine, oxysophocarpine, oxymatrine, matrine, sophoramine, sophoridine and cytisine. Polysaccharides present in seed cell walls are the major inhibitors

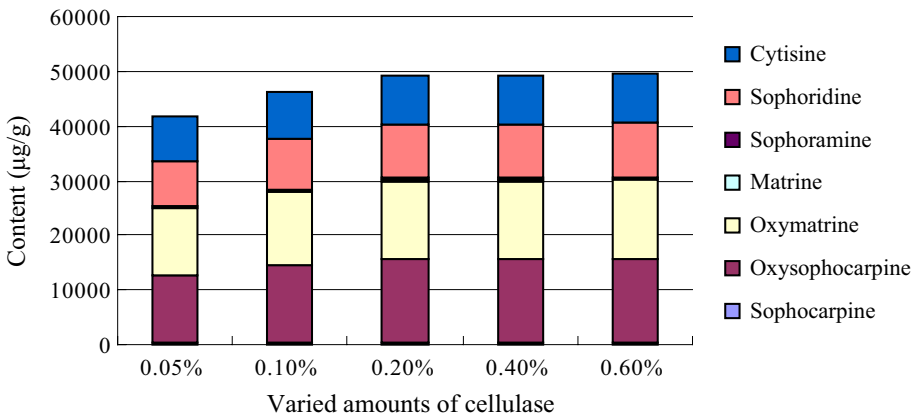

Fig. 2 Effect of the enzyme amount on the alkaloid yield

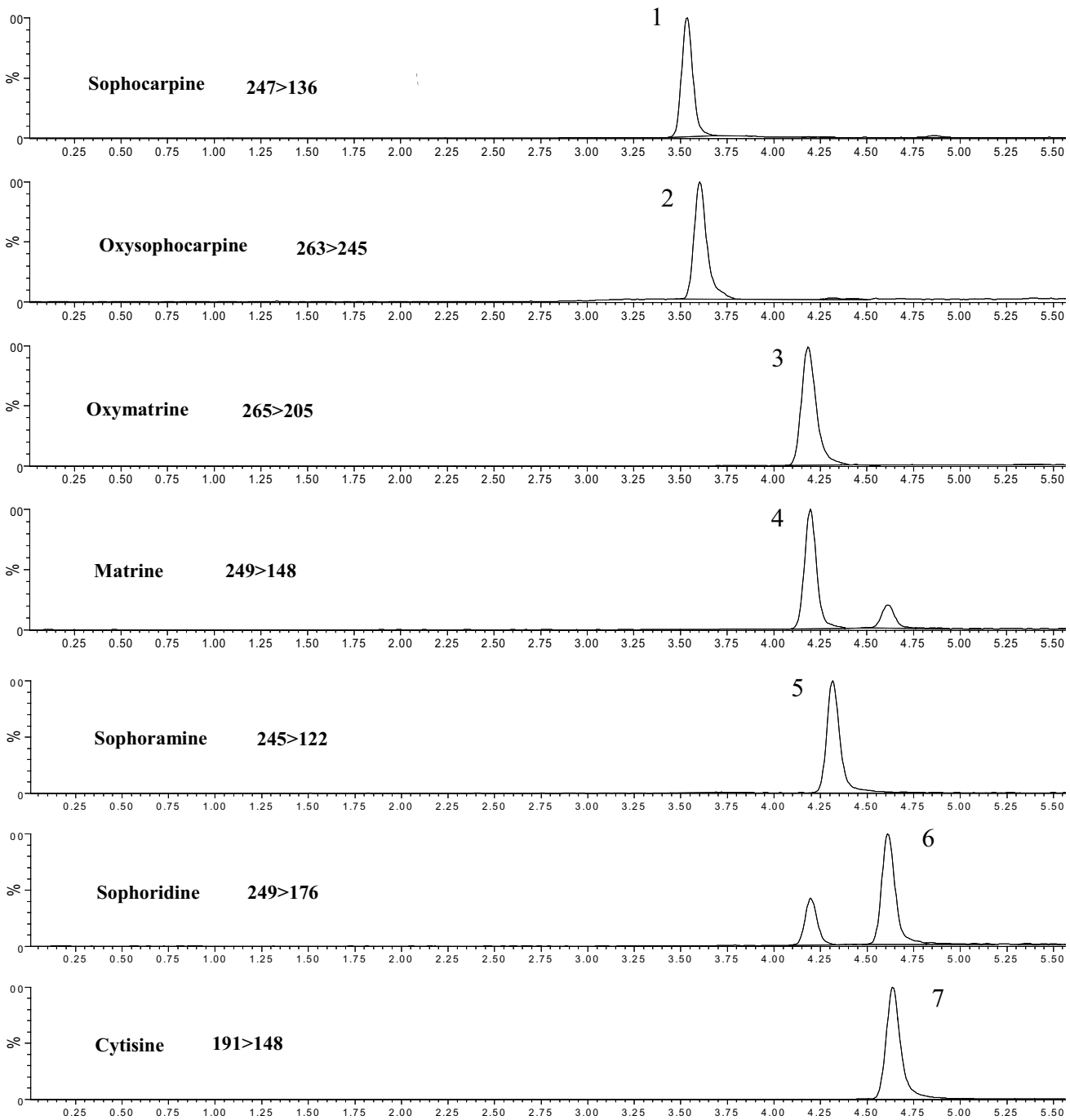

Fig. 3 Chromatogram of the alkaloid MRM transitions analyzed in this study: (1) sophocarpine, (2) oxysophocarpine, (3) oxymatrine, (4) matrine, (5) sophoramine, (6) sophoridine and (7) cytosine

of QAS extraction [22]. To accelerate the release of alkaloids in the UAEH method, the enzyme amount was optimized. The effect of the cellulase level on the alkaloid yield was studied to determine the optimal cellulase amount. As shown in Fig. 2, the alkaloid extraction yield increased with the increasing enzyme amount until reaching an enzyme amount of 0.2%. Increasing the amount of enzyme results in improved degradation of the cell wall. Based on our results, we chose 0.2% as the optimal enzyme concentration.

Fitting the response surface models

The yields of seven alkaloid compounds from *S. alopecuroides* seeds are shown in Table 3. Multiple regression analysis using a quadratic polynomial model [Eq. (1)] was performed using the results in Table 3. The analysis of variance (ANOVA) results and regression coefficients (Supplementary Table 1) indicated that the quadratic model is significant ($p < 0.05$). The "fitness" of the model was investigated using a lack-of-fit test ($p > 0.05$), which indicated the model's ability to accurately predict variations [23].

Effect of the extraction parameters on different alkaloid compounds

Figure 3 shows the chromatogram of MRM transitions for the alkaloids analyzed in this study. Sophocarpine, oxysophocarpine, oxymatrine, matrine, sophoramine, sophoridine and cytisine were identified according to the standards' UHPLC retention times and mass/charge ratios (m/z). The chemical structures of the seven alkaloids investigated are shown in Fig. 1. The chemical properties of these alkaloids vary significantly due to differences in their double bonds and steric configuration. As a result, it is difficult to develop a single optimal procedure for the extraction of all seven alkaloids. Three-dimensional response surface contour plots for these seven compounds as functions of the four independent variables were investigated. The model contains six two-way interactions ($X_1 \times X_2$, $X_1 \times X_3$, $X_1 \times X_4$, $X_2 \times X_3$, $X_2 \times X_4$ and $X_3 \times X_4$).

The three-dimensional response surface contour plots (Supplementary Material Figs. 1–7) depict the yield changes of sophocarpine, oxysophocarpine, oxymatrine, matrine, sophoramine, sophoridine and cytisine as a function of the four independent variables investigated in this study. The conditions for the maximum yield of the seven alkaloids are shown in Table 4. The sophocarpine yield was significantly ($p < 0.05$) affected by the pH, extraction temperature and extraction time, with positive correlation. The effect of pH was higher than that of the other factors. The oxysophocarpine and oxymatrine yields were significantly ($p < 0.05$) affected by the pH and extraction temperature, with positive correlation. For the oxysophocarpine yield, the effect of the extraction temperature was higher than that of the pH, and for the oxymatrine yield, it was the opposite. The matrine yield was significantly ($p < 0.05$) affected by the extraction temperature and extraction time, with positive correlation. The effect of the extraction time was higher than that of the extraction temperature, and the pH had an interaction effect with extraction temperature. The sophoramine yield and cytisine yield were significantly ($p < 0.05$) affected by all the four factors, with positive correlation. For the sophoramine yield, the effect of the extraction temperature was higher than that of the other factors. For the cytisine yield, the effect of the pH was higher than that of the other factors, and pH had an interaction effect with extraction time. The sophoridine yield was significantly ($p < 0.05$) affected by the extraction temperature, extraction time and solvent-to-material ratio, with positive correlation. The effect of the extraction temperature was higher than that of the other factors.

Comparison of different extraction methods

Table 5 compares the yields of the seven alkaloids obtained using different extraction methods (RH, EE, UAE and UAEH). UAEH proved to be superior to EE and UAE in terms of alkaloid yield. The comparatively poor alkaloid yields of the EE and UAE processes encouraged us to investigate the combination of biocatalysis with sonication. The UAEH process improved the alkaloid yield by 33.57% for sophocarpine, 125.31% for oxysophocarpine, 136.17% for oxymatrine, 32.13% for matrine, 43.28% for sophoramine, 69.69% for sophoridine and 70.67% for cytisine compared to the EE method. The UAEH process improved the extraction rate by 15.04% for sophocarpine, 29.29% for oxysophocarpine, 27.59% for oxymatrine, 17.65% for matrine, 6.70% for sophoramine, 28.44% for sophoridine and 19.26% for cytisine compared to the UAE method. Surprisingly, the

Table 4 The conditions for the maximum yield of the seven alkaloids in this study

Alkaloids	pH	Extraction temperature (°C)	Extraction time (min)	Solvent-to-material (mL/g)	Figures
Sophocarpine	5*#	54*	62*	116	S. Figure 1
Oxysophocarpine	5*	55*#	55	100	S. Figure 2
Oxymatrine	5*#	53*	64	115	S. Figure 3
Matrine	5	53*	56*#	105	S. Figure 4
Sophoramine	5*	55*#	70*	131*	S. Figure 5
Sophoridine	5	55*#	59*	113*	S. Figure 6
Cytisine	5*#	53*	69*	118*	S. Figure 7

* Means the factor significantly ($p < 0.05$) affected the yield

#Means the factor's effect was higher than that of the other factors

Table 5 Predicted and experimental values for the seven alkaloid compounds obtained via UAEH, UAE, EE and RH extraction with the corresponding extraction conditions

Alkaloids	Extraction methods								Predicted values
	UAE[a]	EE (2 h)[a]	EE (4 h)[a]	EE (6 h)[a]	EE (8 h)[a]	EE (10 h)[a]	RH[a]	UAEH[a]	
Sophocarpine	361.87 ± 7.58	148.39 ± 2.96	219.39 ± 4.81	255.15 ± 5.15	305.83 ± 7.62	311.65 ± 7.46	5876.25 ± 182.15	410.46 ± 10.58	416.46
Oxysophocarpine	11669.99 ± 373.40	3223.61 ± 93.47	4824.65 ± 154.36	5303.27 ± 185.65	6532.59 ± 209.02	6697.19 ± 227.69	4972.66 ± 169.04	15377.73 ± 525.79	15174.73
Oxymatrine	11345.41 ± 385.73	3110.54 ± 111.96	4398.16 ± 171.52	5023.35 ± 175.80	6029.89 ± 229.102	6129.50 ± 214.65	5696.26 ± 216.44	14635.47 ± 548.61	14500.47
Matrine	354.91 ± 12.98	142.38 ± 5.53	208.05 ± 7.28	260.01 ± 8.32	302.62 ± 10.57	316.42 ± 10.11	6063.64 ± 254.64	422.18 ± 15.94	418.34
Sophoramine	296.83 ± 10.06	98.32 ± 3.13	155.49 ± 5.01	178.78 ± 4.98	216.53 ± 7.27	221.03 ± 7.53	299.11 ± 11.36	325.36 ± 9.85	322.36
Sophoridine	7728.68 ± 309.12	2871.73 ± 117.71	4085.49 ± 183.82	4840.46 ± 193.68	5745.63 ± 224.05	5850.13 ± 244.83	7150.96 ± 300.33	9864.06 ± 456.23	9933.06
Cytisine	7829.67 ± 242.69	2688.18 ± 69.88	3797.07 ± 121.50	4783.29 ± 133.92	5298.85 ± 174.83	5471.04 ± 170.98	7703.60 ± 238.79	9222.39 ± 333,76	9356.39

[a]Mean ± standard deviation ($n = 5$)

matrine and sophocarpine yields from the RH method were 6064.64 and 5876.26 μg/g, respectively, approximately 14–19-fold higher than the matrine and sophocarpine yields from the other three methods. However, the oxymatrine and oxysophocarpine yields from the RH method were only 5696.26 and 4972.66 μg/g, respectively. These results suggest that oxymatrine and oxysophocarpine degrade to matrine and sophocarpine during high temperature extraction, confirming the work conducted by Pan et al. [11]. These results demonstrate the potential of UAEH as an alternative method for alkaloid extraction from *S. alopecuroides* seeds.

Verification of predictive models

Based on our experimental results, a study was performed to evaluate the optimal extraction parameters for a combination of the seven alkaloid compounds investigated in this study. The RSM-guided optimization determined the optimal treatment conditions to be a pH of 5.12, an extraction temperature of 53.96 °C, an extraction time of 59.74 min and a solvent-to-material ratio of 112.01 mL/g. As shown in Table 5, the predicted values were not significantly different ($p > 0.05$) from the experimental values, as determined by a paired *t* test. Therefore, the predictive performance of the established model is acceptable.

Conclusions

The use of ultrasound energy to facilitate enzymatic hydrolysis was found to improve the extraction of alkaloids from *S. alopecuroides* seeds. RSM was used to optimize the extraction parameters for a combination of seven alkaloid compounds. Compared to UAE and EE extraction, the UAEH process enhanced the extraction yield and shortened the extraction time. In addition, the UAEH process can be operated under mild conditions, resulting in lower ingredient degradation compared to the RH method. Catalyzed hydrolysis combined with cavitation was shown to be a highly efficient and environmentally friendly method for the extraction of alkaloids from *S. alopecuroides* seeds.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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