

## Article

# Optimization of Steam Distillation Process and Chemical Constituents of Volatile Oil from *Angelicae sinensis* Radix

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**Abstract:** In this study, the steam distillation process of volatile oil from *Angelicae sinensis* Radix was optimized according to the concept of quality-by-design. A homemade glass volatile oil extractor was used to achieve better cooling of the volatile oil. First, the soaking time, distillation time, and liquid–material ratio were identified as potential critical process parameters by consulting the literature. Then, the three parameters were investigated by single factor experiments. The volatile oil yield increased with the extension in the distillation time, and first increased and then decreased with the increase in soaking time and liquid–material ratio. The results confirmed that soaking time, distillation time, and liquid–material ratio were all critical process parameters. The kinetics models of volatile oil distillation from *Angelicae sinensis* Radix were established. The diffusion model of spherical particle was found to be the best model and indicated that the major resistance of mass transfer was the diffusion of volatile oil from the inside to the surface of the medicinal herb. Furthermore, the Box–Behnken experimental design was used to study the relationship between the three parameters and volatile oil yield. A second-order polynomial model was established, with R<sup>2</sup> exceeding 0.99. The design space of the volatile oil yield was calculated by a probability-based method. In the verification experiments, the average volatile oil yield reached 0.711%. The results showed that the model was accurate and the design space was reliable. In this study, 21 chemical constituents of volatile oil from *Angelicae sinensis* Radix were identified by gas chromatograph-mass spectrometer (GC-MS), accounting for 99.4% of the total volatile oil. It was found that the content of Z-ligustilide was the highest, accounting for 85.4%.

**Keywords:** Radix *Angelicae sinensis*; volatile oil; Box–Behnken design; steam distillation; extraction kinetics; design space



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## 1. Introduction

*Angelicae sinensis* Radix is the dry root of *Angelica sinensis* (Oliv.) Diels [1]. It is widely used as a herbal medicine and has the effects of clearing heat and promoting diuresis, invigorating qi, and blood, etc. Volatile oil is an important component of *Angelicae sinensis* Radix [2,3]. The content of neutral oil in the volatile oil of *Angelicae sinensis* Radix is the highest. The volatile oil of *Angelicae sinensis* Radix mainly contains Z-ligustilide and other components [4,5]. Volatile oil is also considered as the main effective component of *Angelicae sinensis* Radix, which has the effects of treating hypertension [6], analgesic and anti-inflammatory [7], and anti-tumor [8]. The preparation process of the *Angelicae sinensis* Radix volatile oil exists in the production of many Chinese medicines such as Danggui Tiaojing granules, Ruhe Sanjie tablets, Compound Herba Leonuri capsules, and Yangxueyin oral liquid, which are all included in the 2020 edition of the Chinese Pharmacopoeia (1st Part).

At present, the reported preparation methods of angelica volatile oil include steam distillation, supercritical CO<sub>2</sub> extraction, organic solvent extraction, microwave assisted extraction, ultrasound assisted extraction, and so on [9]. Microwave assisted extraction has the advantages of rapidity [10], but a high microwave power may lead to a decrease in the yield of essential oil because of the loss of active compounds [9]. Ultrasound assisted extraction has the advantages of less solvent consumption and better efficiency [11], but there are difficulties in scale-up production. In industry, steam distillation is most widely used to obtain angelica volatile oil [4]. Steam distillation has the advantages of simple equipment, low cost, and a safe solvent [12–14]. In the Chinese Pharmacopoeia, there are more than 30 preparations that use the volatile oil of *Angelicae sinensis* Radix, where more than 80% used the steam distillation process. It is also the method used to determine the volatile oil content of drugs in the Chinese Pharmacopoeia.

However, there are some practical problems in the distillation of volatile oil from *Angelicae sinensis* Radix such as low yield, easy emulsification, and long-time consumption, which bring great challenges to the manufacturing processes and the quality control of Chinese medicines containing *Angelicae sinensis* Radix. Therefore, it is necessary to optimize the distillation process of volatile oil from *Angelicae sinensis* Radix.

In recent years, the quality-by-design concept has been widely applied to optimize the pharmaceutical processes of Chinese medicines [15]. Its implementation steps include: defining the critical process parameters (CPPs), establishing quantitative models of CPPs and pharmaceutical process evaluation indices, establishing the design space, determining the quality control strategies, and continuous improvement, etc. [16]. Before building mathematical models, response surface methodology (RSM) is usually used to collect data with small number of experiments [10]. The Box–Behnken design is often used in many studies [17] as it suggests how to select points from a three-level factorial arrangement, which allows for the efficient estimation of the first- and second-order coefficients of the mathematical model [18].

In this work, steam distillation was used to prepare the volatile oil from *Angelicae sinensis* Radix. A volatile oil extractor with enhanced cooling was used to collect more volatile oil. The CPPs of the distillation process were determined, and the distillation kinetic models were established. According to the results, the main mass transfer resistance of the volatile oil distillation was determined. Then, taking the volatile oil yield as the index, the Box–Behnken experimental design was used to study the effects of the distillation time, soaking time, and liquid–material ratio [10]. The design space of the distillation process of volatile oil from *Angelicae sinensis* Radix was obtained with a probability-based method, which was verified by experiments. Finally, the chemical constituents in the volatile oil of *Angelicae sinensis* Radix were determined by GC-MS, and the results were compared with those reported in the literature.

## 2. Materials and Methods

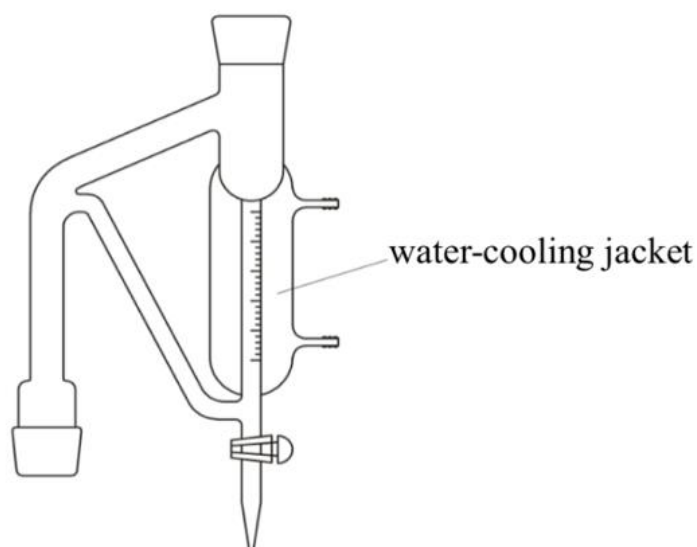
### 2.1. Materials, Reagents, and Instruments

*Angelicae sinensis* Radix was obtained from the Jiangxi Zhangshu Tianqitang Chinese Herbal Pieces Co., Ltd. (batch number: 2009007, origin: Gansu). All the above medicinals were identified by Professor Ge Fei from the Identification Teaching and Research Section of the Jiangxi University of Chinese Medicine. The moisture content of *Angelica sinensis* was  $10.0 \pm 0.5\%$ . Anhydrous sodium sulfate (batch number: 180408) and anhydrous ethanol (batch number: 200707) were purchased from Xilong Scientific Co., Ltd. (Shantou, China). Distilled water was self-made in the laboratory.

### 2.2. Distillation Method

Gansu Province is a genuine producing area of *Angelicae sinensis* Radix. Therefore, the *Angelicae sinensis* Radix from Gansu Province was used in this work. The steam distillation was used to prepare the volatile oil from *Angelicae sinensis* Radix. Figure 1 shows the essential oil extractor. With the homemade essential oil extractor, better cooling could

be achieved. Compared to that of using a conventional extractor, more essential oil can be extracted.



**Figure 1.** Homemade essential oil extractor.

A proper amount of *Angelicae sinensis* Radix was taken and then crushed with a crusher (FD200T, Shanghai Traditional Chinese Medicine Machinery Factory, Shanghai, China). A total of 100 g of *Angelicae sinensis* Radix powder was taken and added into a 2000 mL flask. Water and several glass beads were added into the flask. After that, the flask was shaken to wet the herbal powders and was connected with a condenser tube. Before the experiment, water was added into the volatile oil extractor. An electric heating jacket (KDM type, Shandong Zhencheng Hualu Electric Equipment Co., Ltd., Qingdao, China) was used to heat the flask. After boiling, the electric heating jacket was adjusted to keep boiling. The heating was stopped after a certain time of distillation. After more than 1 h, the height of the volatile oil was measured with a vernier caliper (0–150 mm, 3V type, Guilin Ganglu Digital Measurement and Control Co., Ltd., Guilin, China). The volume of volatile oil was calculated, then the volatile oil was collected. The obtained volatile oil was dehydrated and dried with anhydrous sodium sulfate overnight to obtain an oil-like product until the amount of volatile oil no longer increased. The *Angelicae sinensis* Radix volatile oil was sealed in brown reagent bottles and stored in a refrigerator at 4 °C. The volatile oil yield was calculated using Equation (1).

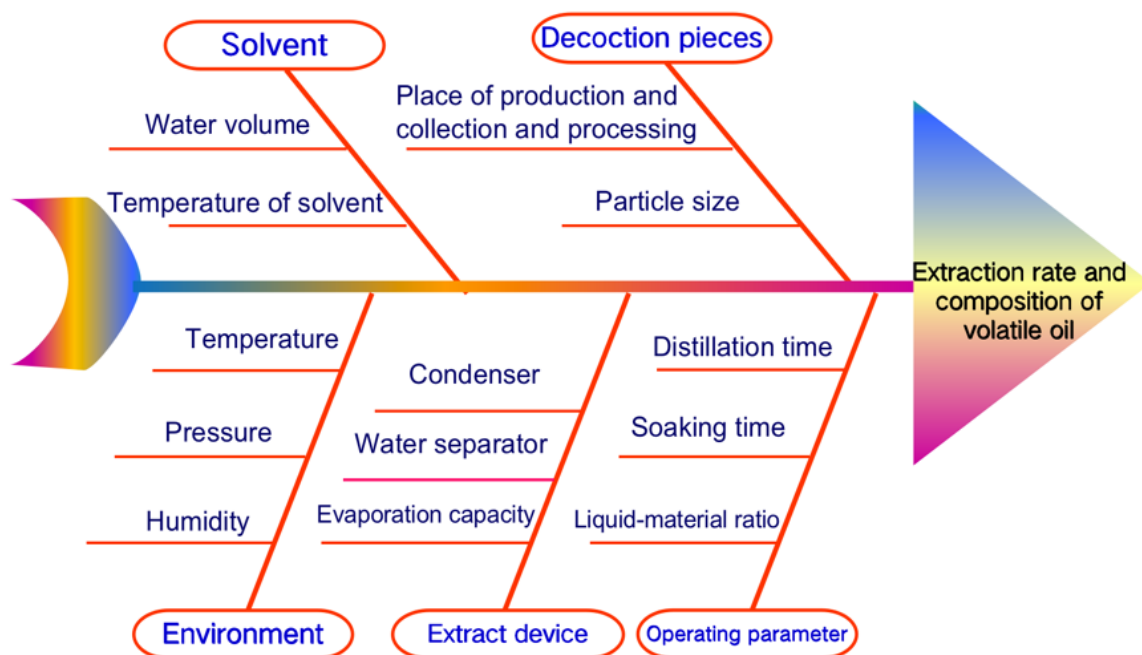
$$\text{Volatile oil yield (\%)} = [\text{volume of oil (mL)}/\text{weight of decoction pieces (g)}] \times 100\% \quad (1)$$

### 2.3. Optimization of Distillation Process Parameters

After the literature review, the process parameters of distilling the volatile oil from herbal medicines by steam distillation were found and are shown in an Ishikawa diagram (Figure 2).

According to the pre-experiments, it was considered that the potential CPPs affecting the volatile oil yield by steam distillation were distillation time (A), soaking time (B), and the liquid–material ratio (C). In this study, the above three factors were investigated by single factor experiments. During the investigation, a total of 100 g of *Angelicae sinensis* Radix powder was taken, soaked for a certain time, and then heated to distill the volatile oil. After reaching the distillation time, the heating was stopped. After the volatile oil was cooled, the volume of the volatile oil was accurately read, and the volatile oil yield was calculated. When the distillation time was studied, the liquid–material ratio was 10:1 mL·g<sup>−1</sup> and the soaking time was 2 h. When the soaking time was studied, the ratio of the liquid to solid

was 10:1 mL·g<sup>-1</sup> and the distillation time was 4 h. When the liquid–material ratio was studied, the distillation time was 4 h and the soaking time was 2 h.



**Figure 2.** The Ishikawa diagram of the steam distillation process for volatile oil distillation.

Next, the Box–Behnken design was used to optimize the distillation process of the volatile oil from *Angelicae sinensis* Radix with the distillation time, soaking time, and liquid–material ratio as the factors. Compared with some other commonly used response surface designs, the number of experiments in the Box–Behnken design is relatively smaller when there are three 3-level factors. For example, there are 29, 17, and 13 runs for the full factorial design, central composite design, and the Box–Behnken design when there are three repetitions of the center point, respectively. The volatile oil yield was considered as the evaluation index. The factor levels are shown in Tables 1 and 2.

**Table 1.** Factors and levels of the Box–Behnken design.

Factor	Level		
	Low (−1)	Medium (0)	High (1)
A: Distillation time (h)	6	8	10
B: Soaking time (h)	2	3	4
C: Liquid–material ratio (mL·g <sup>-1</sup> )	6:1	10:1	14:1

**Table 2.** The Box–Behnken designed experiments and results.

Serial Number	A (Distillation Time/h)	B (Soaking Time/h)	C [Liquid-Material Ratio (mL·g <sup>-1</sup> )]	Y (Volatile Oil Yield/%)
1	−1	−1	0	0.58
2	1	−1	0	0.63
3	−1	1	0	0.59
4	1	1	0	0.65
5	−1	0	−1	0.55
6	1	0	−1	0.61
7	−1	0	1	0.52
8	1	0	1	0.56
9	0	−1	−1	0.61

Table 2. Cont.

Serial Number	A (Distillation Time/h)	B (Soaking Time/h)	C [Liquid-Material Ratio (mL·g <sup>-1</sup> )]	Y (Volatile Oil Yield/%)
10	0	1	−1	0.65
11	0	−1	1	0.56
12	0	1	1	0.57
13	0 (8 h)	0 (3 h)	0 (10:1)	0.70
14	0 (8 h)	0 (3 h)	0 (10:1)	0.71
15	0 (8 h)	0 (3 h)	0 (10:1)	0.71
16	0 (8 h)	0 (3 h)	0 (10:1)	0.72
17	0 (8 h)	0 (3 h)	0 (10:1)	0.71

2.4. Analysis of Chemical Constituents of Volatile Oil from *Angelicae sinensis Radix*

A total of 100 μL of volatile oil was measured accurately, diluted to 10 mL with ethanol, filtered with a 0.22 μm microporous filter membrane, and loaded into a sample bottle. The chemical constituents of the volatile oil samples were determined by gas chromatography-mass spectrometry (Agilent 7890A/5975C, Agilent, Santa Clara, CA, USA).

The gas chromatographic conditions were as follows [19]: HP-5MS capillary quartz column (30 m × 250 μm × 0.25 μm); injection volume: 1.0 μL; the inlet temperature: 230 °C; no diversion; heating procedure: 80 °C, keeping for 0 min; heating at 3 °C min<sup>-1</sup> to 167 °C for 2.5 min, at 2 °C min<sup>-1</sup> to 180 °C for 0 min, at 30 °C min<sup>-1</sup> to 280 °C for 1 min; the transmission line temperature: 300 °C; the carrier gas was high purity helium, the flow rate was 1 mL min<sup>-1</sup>, and the injection volume was 1 μL. Mass spectrometry conditions: Ion source temperature: 230 °C, ionization source was EI, electron energy: 70 eV; the quadrupole temperature: 150 °C, and the scanning quality range was 30–550 amu.

The identification of volatile compounds was performed by computer matching their mass spectra with those stored in a digital library of mass spectral data (NIST 14). The identification results were tentatively identified by the EI-MS spectrum and further experiments are planned to confirm their identification by authentic standards.

2.5. Data Processing Method

2.5.1. Kinetic Models

Volatile oil yield was fitted by Equations (2)–(4), as shown below.

First-order kinetic model:

$$C = C_{eq} (1 - e^{-kt}) \tag{2}$$

where  $C_{eq}$  is the concentration of solution at equilibrium;  $k$  is the total distillation rate constant; and  $t$  is time.

Peleg’s model:

$$C = \frac{t}{(k_1 + k_2t)} \tag{3}$$

where  $k_1$  is the mass transfer rate constant and  $k_2$  is the concentration of the solution at equilibrium.

The diffusion model of spherical particle:

$$C = C_{eq} \left[ 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{Dn^2\pi^2t}{R^2}\right) \right] \tag{4}$$

where  $D$  is the solute diffusion coefficient in the solvent and  $R$  is the particle radius. In the calculation, only the first three terms ( $n = 3$ ) were taken for the sum of the infinite order.

In order to evaluate the fitting effect of each model,  $R^2$  can be calculated according to Equation (5).

$$R^2 = 1 - \frac{\sum(C_{act} - C_{fit})^2}{\sum(C_{act} - \overline{C_{act}})^2} \tag{5}$$

where  $C_{act}$  is the experimental value;  $\overline{C_{act}}$  is the average value of the measured value, and  $C_{fit}$  is the model fitting value. The software MATLAB R2019b (American Math Works Company) was used to analyze and process the data. Except for  $R^2$ , other indices of  $RMSE$ ,  $MSE$ ,  $SSE$ , and  $MAE$  were also calculated according to Equations (6)–(9).

$$RMSE = \sqrt{\frac{\sum(C_{act} - C_{fit})^2}{n}} \tag{6}$$

$$MSE = \frac{\sum(C_{act} - C_{fit})^2}{n} \tag{7}$$

$$SSE = \sum(C_{act} - C_{fit})^2 \tag{8}$$

$$AE = \frac{\sum|C_{act} - C_{fit}|}{n} \tag{9}$$

The software Microsoft Office Excel (American Microsoft Company) was used to analyze and process the data.

### 2.5.2. Data Processing of Volatile Oil Distillation Rate Obtained from Experimental Design

The software “Design-Expert 8.0.6” was used to analyze the experimental data collected from the Box–Behnken designed experiments. The volatile oil yield was taken as the evaluation index (Y), and a second-order polynomial equation fitting was carried out. Equation (10) was adopted as the mathematical model between the evaluation index and the three process parameters.

$$Y = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2 \tag{10}$$

where  $b_{1-9}$  is the partial regression coefficient and  $b_0$  is the intercept.

### 2.5.3. Optimization of Distillation Parameters of Volatile Oil by the Monte Carlo Method

The design space was calculated by the Monte Carlo method with a parameter optimization software compiled by MATLAB R2018b (Math Works Company of America) [20]. According to previous work, the design space calculated with this method is more reliable [20]. Three factors affecting the volatile oil yield were simulated randomly. The combination of parameters with the probability of no less than 0.80 of attaining the preset volatile oil yield value was taken as the design space. In the calculation, the step lengths of distillation time, soaking time, and liquid—material ratio were set to 0.04, 0.02, and 0.08, respectively. The simulations were conducted 2000 times.

## 3. Results and Discussion

### 3.1. Critical Process Parameters of Volatile Oil Distillation

The distillation time was changed to 2, 4, 6, 8, and 10 h, respectively, and the volatile oil yield is shown in Figure 3. The volatile oil yield increased continuously within the first 8 h of distillation time. When the distillation time reached 8 h, the volatile oil yield reached 0.68%, and the yield tended to be stable when the distillation time was prolonged.

The soaking time was changed to 0, 1, 2, 3, and 4 h, the yield of volatile oil from *Angelicae sinensis* Radix is shown in Figure 4. When the soaking time was 3 h, a maximum oil yield of 0.67% was obtained. After soaking time reached 3 h, the oil yield decreased with the extension in the soaking time. The reason may be that the aqueous extract became

viscous when soaking for too long, which was not conducive to the distillation of the volatile oil.

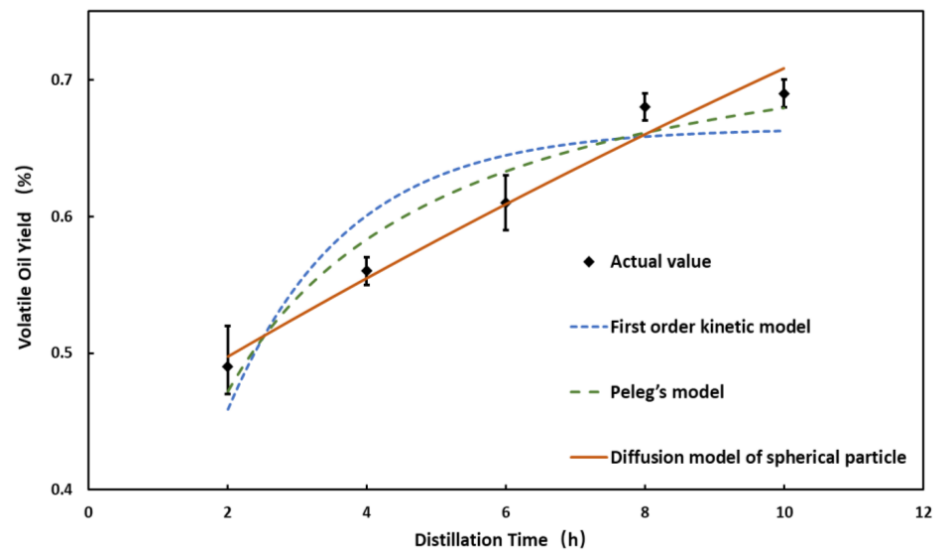


Figure 3. The effect of distillation time on the volatile oil yield ( $n = 3$ ).

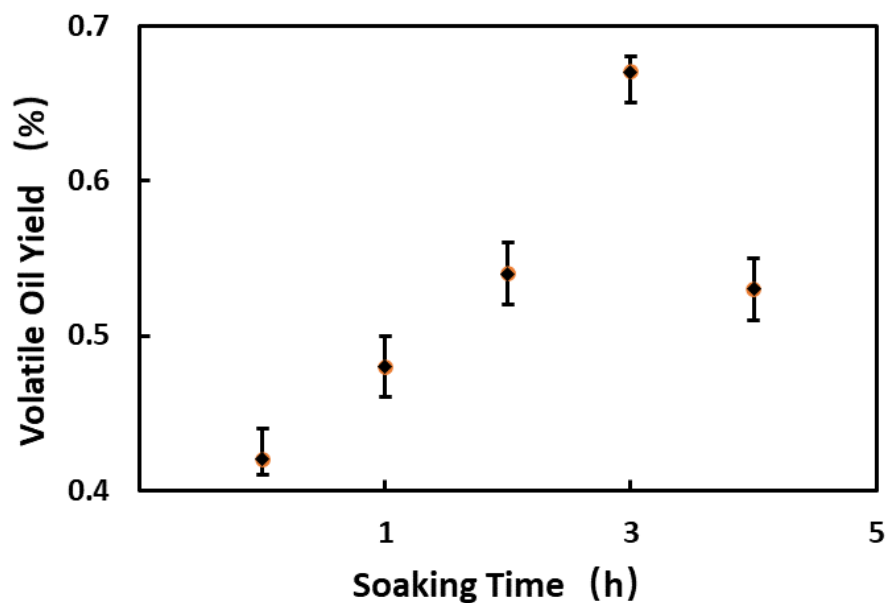


Figure 4. The effect of soaking time on the volatile oil yield ( $n = 3$ ).

The liquid–material ratio was changed to 6:1, 8:1, 10:1, 12:1, and 14:1 ( $\text{mL}\cdot\text{g}^{-1}$ ), respectively, and the volatile oil yield is shown in Figure 5. When the liquid–material ratio was not higher than 10:1, the volatile oil yield increased as the liquid–material ratio increased. The reason may lie in the more uniform heating when the liquid–material ratio increased, which was beneficial to the diffusion and dissolution of the volatile oil. In Figure 4, when the liquid–material ratio was greater than 10:1, the volatile oil yield showed an obvious downward trend, which may be due to the loss caused by the dissolution of the volatile oil in water.

The above results indicate that the distillation time, soaking time, and liquid–material ratio all had a great influence on the yield of the volatile oil from *Angelicae sinensis* Radix and were all indeed the CPPs.

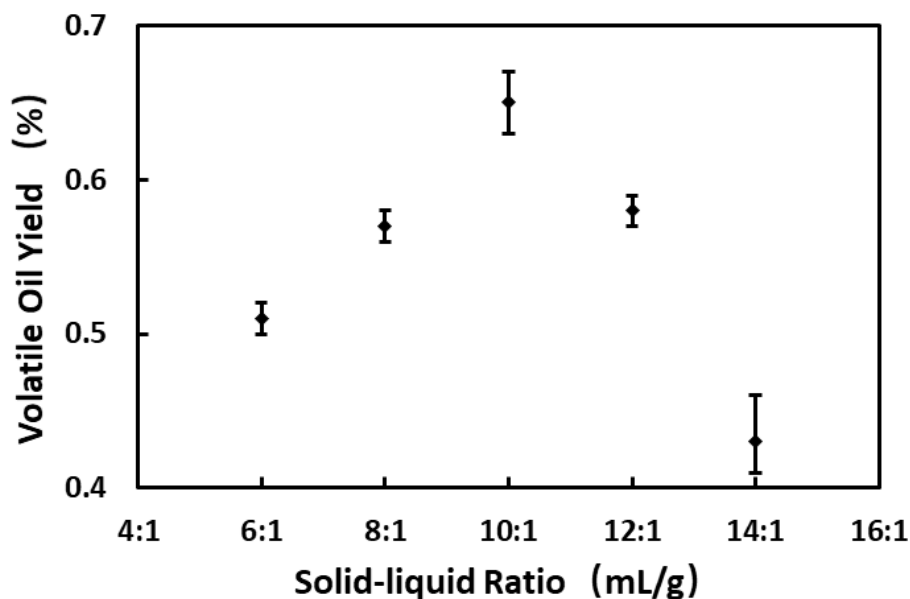


Figure 5. The effect of solid–liquid ratio on the volatile oil yield ( $n = 3$ ).

### 3.2. Study on Kinetics of Volatile Oil Distillation

Three models were used to fit the kinetic data of the volatile oil distillation, and the fitting results are shown in Figure 2. The first-order kinetic model fitting results  $C_{eq}$  and  $k$  were 0.6644% and  $0.5861 \text{ h}^{-1}$ , respectively, and  $R^2$  was 0.8198. The Peleg’s model fitted  $k_1$  and  $k_2$  were  $1.6156 \text{ h} \cdot \%^{-1}$  and  $1.3105 \%^{-1}$ , respectively, and the  $R^2$  was 0.9338. The diffusion model of spherical particle fitted  $C_{eq}$  and  $\frac{D}{R^2}$  were 2.5317% and  $0.0007 \text{ h}^{-1}$ , respectively, and the  $R^2$  was 0.9710. Among the three models, the diffusion model of spherical particle fitted  $R^2$  was the highest, which could explain more than 97% of the variance in experimental data.

Table 3 shows the errors between the predicted values and the actual values of different models. The prediction values of the diffusion model were closest to the actual values.

Table 3. The error results of the different functions.

Model	RMSE ( $10^{-2} \text{ mL/g}$ )	SSE ( $10^{-4} \text{ mL}^2/\text{g}^2$ )	MSE ( $10^{-4} \text{ mL}^2/\text{g}^2$ )	MAE ( $10^{-2} \text{ mL/g}$ )
First-order kinetic model	0.032	0.0051	0.0010	0.031
Peleg’s model	0.019	0.0019	0.00037	0.019
Diffusion model of spherical particle	0.013	0.00082	0.00016	0.010

The diffusion model of spherical particle assumed that the major resistance of mass transfer in the distillation process of volatile oil was the diffusion of volatile oil from the inside to the surface of the medicinal materials. The mass transfer rate of volatile oil from the surface of the medicinal materials to the extraction solution and the mass transfer rate in the air inside the extractor were all much faster. These results indicate that a rapid distillation of volatile oil can probably be realized by lowering the average particle size of *Angelicae sinensis* Radix.

### 3.3. Optimization of Distillation Parameters of Volatile Oil

#### 3.3.1. Data Processing and Model Fitting

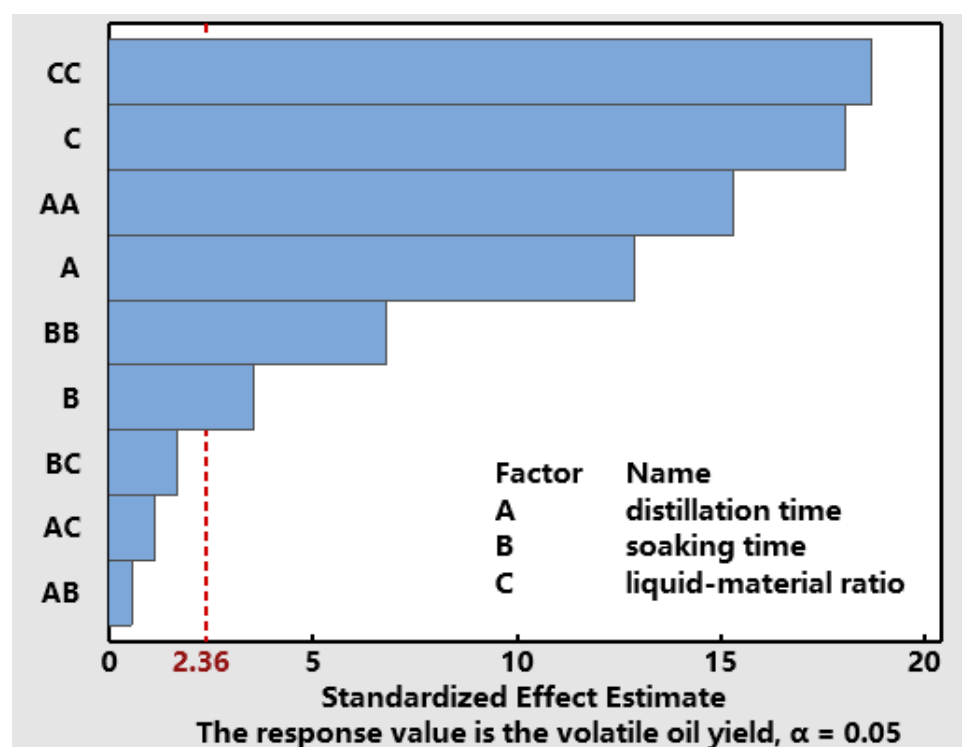
By modeling the oil yield data, the multivariate binomial regression model obtained was as follows:  $Y = 0.710 + 0.026A + 0.010B - 0.026C + 2.50 \times 10^{-3}AB - 5.00 \times 10^{-3}AC - 7.50 \times 10^{-3}BC - 0.068A^2 - 0.030B^2 - 0.082C^2$ . The model determination coefficient



$R^2$  was 0.9917. The variance analysis of each item in the model is shown in Table 4. The F value of the model was 93.13, and  $p < 0.0001$ , which showed that the model was extremely significant. The model  $R^2$  exceeded 0.99, which showed that the model could well explain the data variation in the experiment. A ( $p < 0.0001$ ) and C ( $p < 0.0001$ ) were extremely significant, and B ( $p < 0.05$ ) was significant. Among the quadratic terms,  $A^2$  ( $p < 0.0001$ ),  $B^2$  ( $p = 0.0003$ ), and  $C^2$  ( $p < 0.0001$ ) were all extremely significant terms, which showed that the influence of the three parameters on the oil yield was nonlinear. The results were consistent with the previous single factor experimental results. The  $p$  values of AB, BC, and AC were all greater than 0.10, which showed that the interaction between the three factors was small. Figure 6 shows the Pareto chart. It can be concluded that all the linear terms and quadratic terms were significant.

**Table 4.** The results of the analysis of variance.

Variance Source	Sum of Square	Degree of Freedom	Mean Square	F Value	$p$ Value
Model	0.069	9	$7.65 \times 10^{-3}$	93.13	<0.0001
A	$5.513 \times 10^{-3}$	1	$5.513 \times 10^{-3}$	67.11	<0.0001
B	$8 \times 10^{-4}$	1	$8 \times 10^{-4}$	9.74	0.0168
C	$5.512 \times 10^{-3}$	1	$5.512 \times 10^{-3}$	67.11	<0.0001
AB	$2.5 \times 10^{-5}$	1	$2.5 \times 10^{-5}$	0.3	0.5983
AC	$1 \times 10^{-4}$	1	$1 \times 10^{-4}$	1.22	0.3064
BC	$2.25 \times 10^{-4}$	1	$2.25 \times 10^{-4}$	2.74	0.1419
A22	0.019	1	0.019	233.55	<0.0001
B22	$3.789 \times 10^{-3}$	1	$3.789 \times 10^{-3}$	46.13	0.0003
C22	0.029	1	0.029	348.88	<0.0001
Residual error	$5.75 \times 10^{-4}$	7	$8.214 \times 10^{-5}$		
Misfit term	$3.75 \times 10^{-4}$	3	$1.25 \times 10^{-4}$	2.5	0.1985
Pure error	$2 \times 10^{-4}$	4	$5 \times 10^{-5}$		
Total error	0.069	16			



**Figure 6.** The Pareto chart of the parameters.

### 3.3.2. Response Surface Diagram and Contour Diagram

The response surface diagram and contour diagram of the distillation process of the volatile oil from *Angelicae sinensis* Radix are shown in Figure 7. The figure reflects the effects of the distillation time, soaking time, and liquid–material ratio on the oil yield. When the liquid–material ratio is fixed, the oil yield increases first and then decreases gradually with the increase in distillation time. When the distillation time is too long, some volatile components in the volatile oil may volatilize and be lost. When the soaking time is fixed, the volatile oil yield increases first and then decreases gently with the increase in the distillation time, and increases first and then decreases with the increase in the liquid–material ratio. When the distillation time is fixed, the oil yield increases first and then stabilizes with the increase in soaking time, and increases first and then decreases with the increase in the liquid–material ratio.

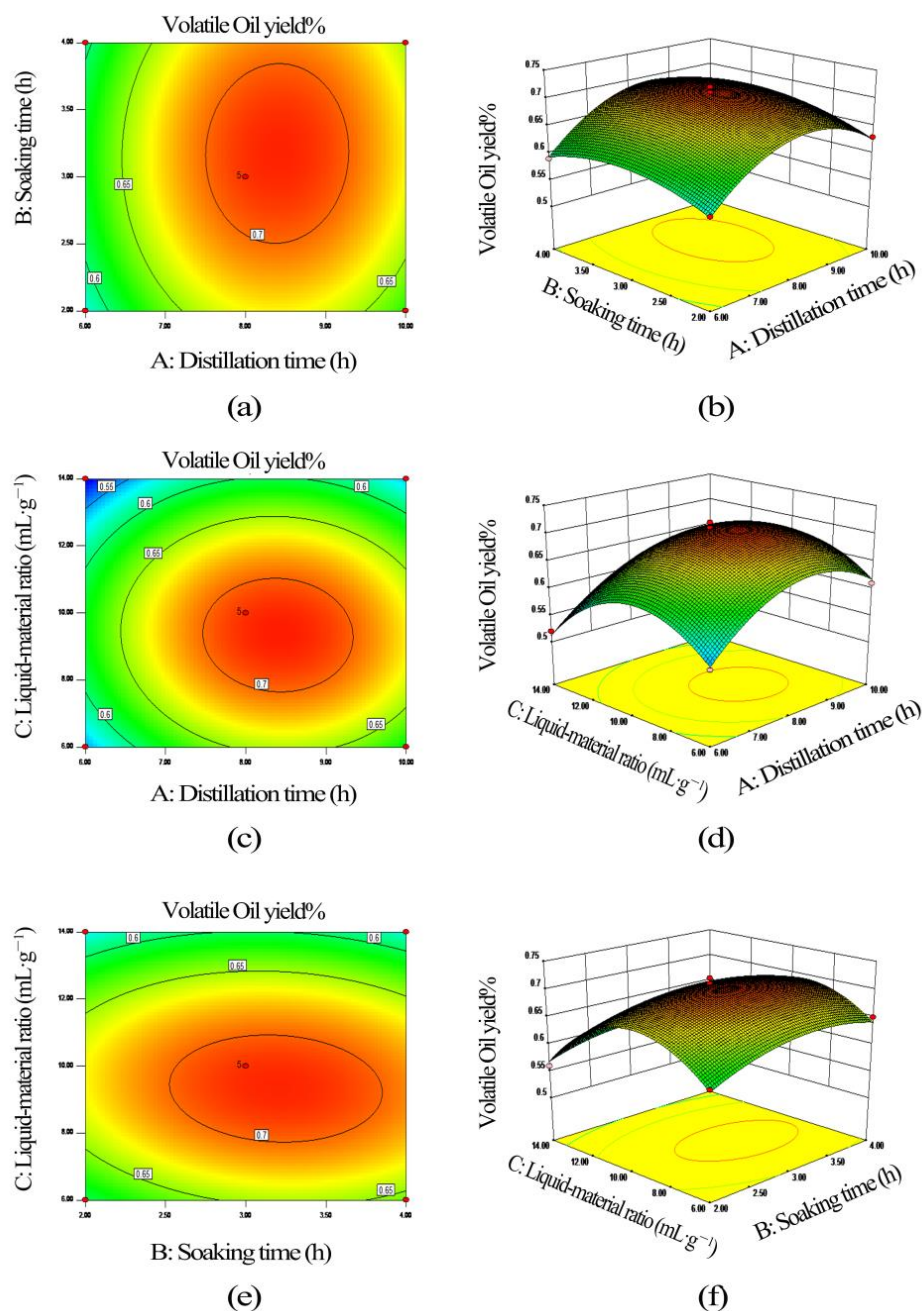
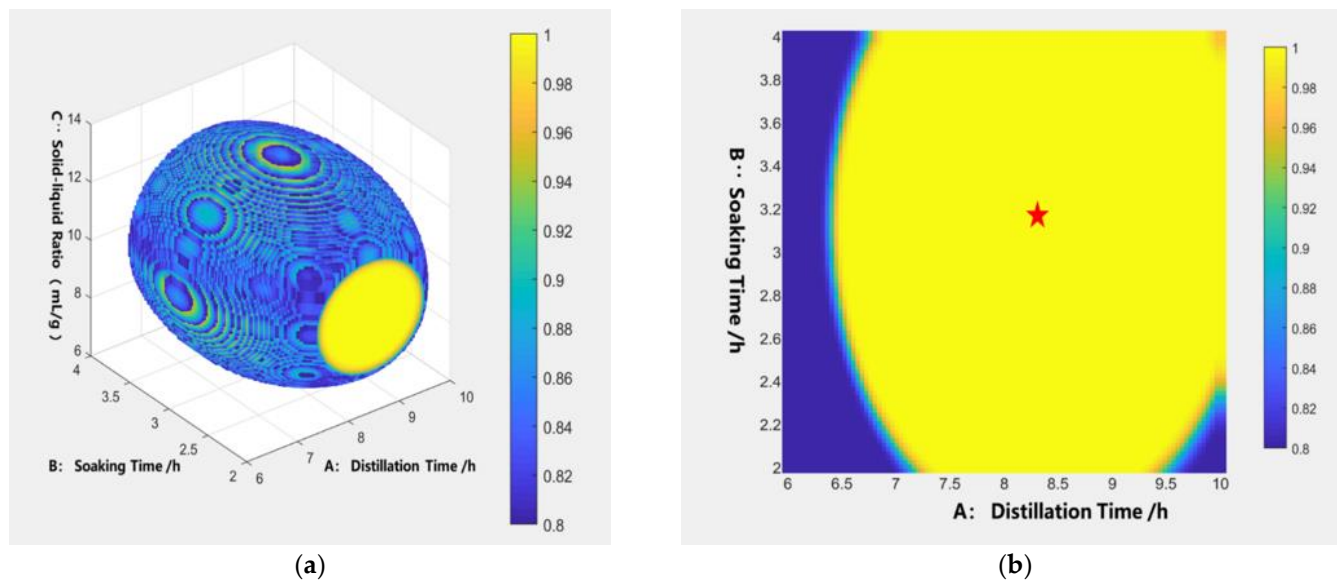


Figure 7. The 3D response surface and 2D contour map for the distillation process of volatile oil.

### 3.3.3. Design Space Calculation and Verification

The acceptable lower limit of volatile oil yield was set at 0.65%, and the lowest probability of target attainment was set at 0.80. The design space calculated by the probability-based method is shown in Figure 7. Figure 7a is a three-dimensional design space diagram, and Figure 8b is a two-dimensional space diagram after fixing the liquid–material ratio at 9.3:1. It can be seen from the figure that the design space was close to the shape of a football.



**Figure 8.** The design space. (a) Panorama of the design space. (b) The design space when the liquid–material ratio = 9.3:1 ( $\text{mL}\cdot\text{g}^{-1}$ ). The red star in the figure represents the condition of the verification experiments, and the color bar indicates the probability of the target attainment.

The design space was verified with a selected condition of 8.4 h of distillation time, 3.2 h of soaking time, and 9.3:1 ( $\text{mL}\cdot\text{g}^{-1}$ ) of liquid–material ratio. Under this condition, the probability for target attainment was 1.0 and the predicted volatile oil yield was 0.715%. Three parallel validation tests were carried out, and the oil yield of *Angelicae sinensis* Radix volatile oil was 0.711%, 0.709%, and 0.712%, respectively. The average oil yield was 0.711% and the relative standard deviation was 0.21%. The density of the collected volatile oil was  $1.0075 \text{ g}/\text{cm}^3$  at room temperature. The mathematical model was developed according to the results of the Box–Behnken designed experiments. The measured values were close to the predicted values, indicating that the prediction of the model was accurate. The volatile oil yield was higher than the preset standard, which shows that the design space was reliable.

### 3.4. Qualitative Analysis of Chemical Constituents of Volatile Oil

The GC-MS analysis of the volatile oil from *Angelicae sinensis* Radix was carried out, and the total ion flow diagram is shown in Figure 9. The results of the mass spectrometry are shown in Table 5. Twenty chemical constituents were identified, accounting for 99.426% of the total volatile oil. Among them, Z-ligustilide had the highest relative content, and its relative content reached 85.385%. Its structural formula can be found in SciFinder [21]. Other components with relatively high contents were  $\alpha$ -pinene,  $\beta$ -ocimene, 2-methyl-1,3-benzoxazole, butylidenephthalide, and E-ligustilide.

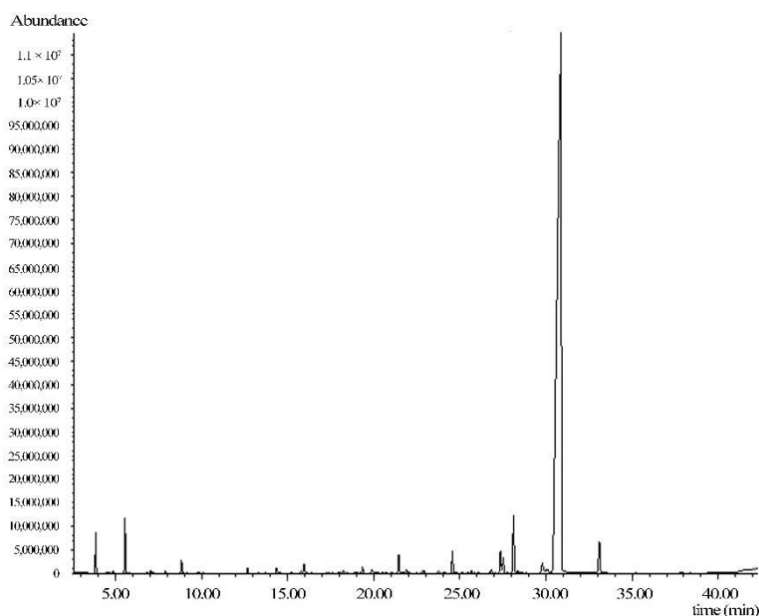


Figure 9. The total ion chromatogram of the angelica volatile oil.

Table 5. The tentative identification results of the chemical composition of angelica volatile oil.

Serial Number	T/min	Compound	Chemical Formula	Relative Content/%	m/z Value of the Fragment with Maximum Abundance	Abundance
		<b>α-Pinene</b>				
1	3.85	2,6,6-trimethylbicyclo [3.1.1]hept-2-ene	C <sub>10</sub> H <sub>16</sub>	1.21	93.1	239,680
		<b>β-Ocimene</b>				
2	5.55	3,7-dimethylocta-1,3,6-triene	C <sub>10</sub> H <sub>16</sub>	1.67	93.1	298,432
3	8.84	6-butylocta-1,4-diene	C <sub>11</sub> H <sub>18</sub>	0.53	79.1	61,936
		<b>Diamyl Ketone</b>				
4	12.68	undecan-6-one	C <sub>11</sub> H <sub>22</sub> O	0.21	71.1	22,096
5	14.33	4-ethenyl-2-methoxyphenol	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	0.30	150.1	32,720
		<b>Duraldehyde</b>				
6	15.95	2,4,5-trimethylbenzaldehyde	C <sub>10</sub> H <sub>12</sub> O	0.46	147.1	34,272
		<b>β-Cedrene</b>				
7	18.24	(1S,2R,5S,7S)-2,6,6-trimethyl-8-methylidenetricyclo [5.3.1.01,5]undecane	C <sub>15</sub> H <sub>24</sub>	0.27	161.2	7463
		<b>Sesquichamene</b>				
8	19.36	4-(1,2-dimethylcyclopent-2-en-1-yl)butan-2-one	C <sub>11</sub> H <sub>18</sub> O	0.25	93.1	12,845
9	19.88	2,4a,8,8-tetramethyl-1,1a,4,5,6,7-hexahydrocyclopropa[j]naphthalene	C <sub>15</sub> H <sub>24</sub>	0.02	93.1	4696
10	21.45	(1R,2R)-1-ethenyl-1-methyl-4-propan-2-ylidene-2-prop-1-en-2-ylcyclohexane	C <sub>15</sub> H <sub>24</sub>	0.75	121.1	50,288
		<b>β-Bisabolene</b>				
11	21.90	(4S)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohexene	C <sub>15</sub> H <sub>24</sub>	0.17	69.1	9638
		<b>Alloaromadendrene</b>				
12	23.77	1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene	C <sub>15</sub> H <sub>24</sub>	0.11	121.1	4412

Table 5. Cont.

Serial Number	T/min	Compound	Chemical Formula	Relative Content/%	m/z Value of the Fragment with Maximum Abundance	Abundance
		<b>Spathulenol</b>				
13	24.57	(7S)-1,1,7-trimethyl-4-methylidene-1a,2,3,4a,5,6,7a,7b-octahydrocyclopropa[h]azulen-7-ol	C <sub>15</sub> H <sub>24</sub> O	0.96	91.1	22,384
14	27.36	2-methyl-1,3-benzoxazole	C <sub>8</sub> H <sub>7</sub> NO	1.06	133.0	240,832
15	27.52	1-oxido-4-[2-(1-oxidopyridin-1-ium-4-yl)ethyl]pyridin-1-ium	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	0.71	108.1	70,000
16	27.75	<b>Cyclopentadiene</b> 2,5,5-trimethylcyclopenta-1,3-diene	C <sub>8</sub> H <sub>12</sub>	0.09	159.0	373,632
17	28.12	<b>Butylidenephthalide</b> (3E)-3-butylidene-2-benzofuran-1-one	C <sub>12</sub> H <sub>12</sub> O <sub>2</sub>	2.79	159.0	56,168
18	30.06	<b>Senkyunolide A</b> (3S)-3-butyl-4,5-dihydro-3H-2-benzofuran-1-one	C <sub>12</sub> H <sub>16</sub> O <sub>2</sub>	0.36	107.1	27,088
19	30.88	<b>Z-ligustilide</b> (3Z)-3-butylidene-4,5-dihydro-2-benzofuran-1-one	C <sub>12</sub> H <sub>14</sub> O <sub>2</sub>	85.4	161.1	1,526,784
20	32.34	<b>E-ligustilide</b> (3E)-3-butylidene-4,5-dihydro-2-benzofuran-1-one	C <sub>12</sub> H <sub>14</sub> O <sub>2</sub>	2.04	161.1	86,504

#### 4. Discussion

Li Tao et al. [22] found that the content of volatile oil from wild *Angelicae sinensis* Radix (1.14%) was more than twice as high than that from the artificially cultivated *Angelicae sinensis* Radix (0.4%). Yan Hui et al. [23] found that longer sunshine was not conducive to the increase in volatile components that mainly consisted of ligustilide. Lin Haiming [24] found that the content of the volatile oil and alcohol-soluble extract decreased gradually with the increase in the drying temperature. Ji Peng et al. [25] determined and analyzed the chemical components of volatile oil in raw *Angelicae sinensis* Radix and different processed products of *Angelicae sinensis* Radix by GC-MS. It was found that different processing methods would affect the total amount of volatile oil in *Angelicae sinensis* Radix and the contents of ligustilide and butenyl phthalolactone in the volatile oil [25]. Li Runhong et al. [26] studied the difference in the volatile oil composition and content in *Angelicae sinensis* Radix from different producing areas. The results showed that the highest volatile oil content of wild *Angelicae sinensis* Radix in Linzhi (Tibet Province) was 2-hydrobutenyl phthalide (70.184%), followed by n-butenyl phthalide (9.288%) [26]. In the volatile oil of wild *Angelicae sinensis* Radix in Pingwu (Sichuan Province), the content of 2-hydrobutenyl phthalide (92.551%) was the highest, followed by butenyl phthalide (3.037%) [26]. These results were different compared with those reported in this study and most of the literature, in which ligustilide was found to be the most abundant constituent. Generally speaking, the composition and content of volatile oil in *Angelicae sinensis* Radix are affected by the growth environment, harvest time, drying method, processing method, extraction technology, etc. [14,27]. Therefore, the composition and content of volatile oil in *Angelicae sinensis* Radix may have significant differences.

The results of some published works [28] have shown that the yield of volatile oil obtained from *Angelica sinensis* by steam distillation was low (about 0.3–0.5%). In industry, the yield is even lower. Sometimes, only the volatile oil aqueous solution can be obtained. In this work, an improved steam distillation with enhanced cooling was used. Compared with that of using a traditional volatile oil extractor, the collected volatile oil could be increased by 30–58%. The collected volatile oil in *Angelica sinensis* was different to that in the literature [22,29,30], which may be attributed to germplasm resources, harvesting

time, planting altitude, processing technology, and so on. However, the efficiency of steam distillation was not high. More volatile oil may be obtained when using other techniques such as supercritical fluid extraction [31].

## 5. Conclusions

In this work, an improved volatile oil extractor with better cooling was applied to collect the volatile oil from *Angelicae sinensis* Radix by steam distillation. The distillation process was optimized according to the concept of quality-by-design. The soaking time, distillation time, and liquid–material ratio were determined as CPPs. It was observed that the volatile oil yield increased with the increase in the distillation time. Furthermore, three models were used to fit the distillation kinetics data of the volatile oil. The fitting effect of the diffusion model of spherical particle was best, and the  $R^2$  exceeded 0.97. This indicates that a more rapid distillation process can probably be realized when smaller *Angelicae sinensis* Radix can be used. The volatile oil yield increased first and then decreased with the increase in the soaking time and liquid–material ratio. Then, the distillation time, soaking time, and liquid–material ratio were investigated with the Box–Behnken designed experiments. The  $R^2$  of the established second-order polynomial model exceeded 0.99. Then, the design space for distilling the volatile oil from *Angelicae sinensis* Radix was obtained with a probability-based method. The design space was verified at a condition shown as follows: Distillation time: 8.41 h, soaking time: 3.20 h, liquid–material ratio: 9.3:1 ( $\text{mL}\cdot\text{g}^{-1}$ ). The probability of the target attainment was 1 at this condition. The actual volatile oil yield was 0.711%, which was close to the predicted value of 0.715% and higher than the preset standard, indicating that the model could predict accurately and the design space was reliable. The volatile oil of *Angelicae sinensis* Radix was also analyzed by GC-MS, and 21 chemical constituents were found, accounting for 99.426% of the total volatile oil. The content of Z-ligustilide in the volatile oil was the highest, and the relative content reached 85.38%.

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