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OPTIMIZATION OF THE EXTRACTION OF TOTAL FLAVONOIDS FROM LAC TIEN (*PASSIFLORA FOETIDA* L.) USING THE RESPONSE SURFACE METHODOLOGY

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Summary

Optimization of the Extraction of Total Flavonoids from Lac Tien (*Passiflora foetida* L.) Using the Response Surface Methodology

This study aims to optimize the extraction conditions of total flavonoids from *Passiflora foetida* L. using the reflux extraction method. The experimental layout is carried out according to the Box - Benhken design method. Using response surface methodology (RSM), an optimal model of the extraction process has been built with two objective functions: extraction efficiency (Y1) and total flavonoid content (Y2); four factors of extraction, which are ethanol concentration, extraction temperature, extraction time and extraction ratio, are studied. Based upon RSM data, the optimal conditions are as follows: ethanol concentration 71.7 (%, v/v), extraction temperature 68.09°C, extraction time 151.18 (minutes), and extraction ratio 25.2/1 (mL/g). At this condition, the extraction efficiency and total flavonoid content in the extract reach the maximum value of 14.95 ± 0.16% and 2.39 ± 0.09%, respectively.

Keywords: *Response Surface Methodology (RSM), Passiflora foetida* L., *Total flavonoid content.*

1. Introduction

Passiflora foetida L., which belongs to the family Passiaceae, is an exotic and fast-growing perennial vine, occurring in many parts of the world in tropical and subtropical regions. In Vietnam, *Passiflora foetida* L. is widely distributed in provinces such as Bac Giang, Lang Son, Cao Bang, Quang Ninh, Thanh Hoa, Nghe An [1],[2]. In Vietnam, the genus Passiflora has 15 species, broadly spread throughout the country from North to South, and some of them are used in traditional medicine. *Passiflora foetida* L. has a sedative effect, helping to treat neurological disorders, insomnia; In addition, it is also found to aid in the treatment of diabetes, asthma, ulcers [3],[4],[5]. In particular, the sedative action and the treatment of insomnia are the most researched interest.

The major phytoconstituents of this plant are flavonoids, alkaloids, phenolics, glycosides, cyanogenic compounds. In particular, flavonoid content has been shown to be associated with sedative and anti-insomnia effects [4][6]. Several flavonoid compounds in *Passiflora foetida* L. have been reported such as pachypodol; 7,4'-dimethoxyapigenin; ermanin; 4,5,7-*O*-dimethyl-naringenin; 3,5-dihydroxy-4,7-dimethoxy flavanone; vitexin; isovitexin; 2"-xylosylvitcxin; luteolin-7-*O*-*β*-D-glucoside [3],[7]. According to Vietnam Pharmacopoeia, the total flavonoid content in *Passiflora foetida* L. was determined to be about 0.6% in dried material. To the best of our knowledge, there has been no research focusing on extracting flavonoid content from *Passiflora foetida* L. Therefore, the aim of this study, which is extracting these active ingredients from *Passiflora foetida* L. has high scientific and practical significance.

Pharmaceutical extraction technology often focuses on increasing extraction efficiency or scalability. In order to improve the quality of crude extracts from medicinal herbs, the factors that affect the quality attributes of the obtained extract need to be optimized. Currently, to replace classical optimization methods, RSM has been widely used in the world. RSM consists of mathematical and statistical techniques used to explore a suitable functional interaction between a response of interest and some process variable [8],[9]. The RSM method has been applied in optimizing conditions for the extraction of natural compounds, chemical synthesis, or optimization of other chemical processes [10],[11]. This shows that the RSM method has an important role in the research field to compare with other methods, helping to improve the efficiency of the process by optimizing the process parameters. In this study, RSM has been optimized for the factors for the flavonoid extraction from *Passiflora foetida* L., contributing to creating a standardized extract for further experimental studies.

2. Materials and Methods

2.1. Plant Sample Preparation

Passiflora foetida L. was collected in April 2022 in Thanh Hoa Province, Vietnam. Samples were collected and identified by the Medicinal Material Resource Center, National Institute of Medicinal Materials. The samples were stored at the Department of Extraction Technology, National of Medicinal Material. Stems and leaves of *Passiflora foetida* L. were dried, crushed, and sieved through a 2mm sieve to obtain samples of uniform size, then sealed in vacuum bags and stored at -5 ^{o}C before proceeding to the next steps. The value of moisture content was kept under 10% before further experiments.

2.2. Method

Extraction method: For each experiment, 10 g of the material was put into a 500 mL three-neck flask. Different volumes of solvents were added into the flask based on the experiment. Install a reflux condenser. Turn on the hotplate to start the extraction process. The extraction time is calculated from the time of heating. At the end of the extraction process, the extract was filtered through filter paper and then concentrated by a vacuum rotary evaporator to collect the crude extract.

Determination of Extraction Yield*:* The yield of flavonoid content was calculated as follows (1):

$$\text{The yield (\%)} = \frac{W_1}{W_2} \times 100 \quad (1)$$

W1 (g) and W2 (g): The mass of the crude extract and the raw material, respectively.

Determination of Total Flavonoid Content (TFC): Determination of total flavonoid content was executed according to the monograph *Passiflora foetida* L., Vietnamese Pharmacopoeia V [12]. Take exactly 5 mL of the crude extract into a beaker, and evaporate in a water bath until dry. Use 10 mL of a mixture of methanol-glacial acetic acid (10:100) to dissolve and transfer the entire sample to a 25 mL volumetric flask, then add 10 mL of a mixture containing 2.5% boric acid and 2% oxalic acid in anhydrous formic acid into the flask as well. Add anhydrous acetic acid to the 25 mL mark and mix well. Blank solution: Take exactly 5 mL of the crude extract into a beaker, and evaporate until dry. Use 10 mL of the methanol-glacial acetic acid (10:100) mixture to dissolve and transfer the entire sample to a 25 mL volumetric flask, then add 10 mL of anhydrous formic acid into the flask as well. Add anhydrous acetic acid to the 25 mL mark and mix well. After 30 min, measure the absorbance of these solutions at 401 nm in a 1 cm cuvette. Calculate the percentage of total flavonoid content (by calculating the Vitexin content) by absorbance A (1%, 1 cm). Take 628 as the value of A (1%, 1 cm) of Vitexin at 401 nm.

Experimental Design: Using the RSM to optimize the extraction conditions of total flavonoids from *Passiflora foetida* L. based on the reflux extraction method. Four parameters of the extraction process were studied including ethanol solvent concentration (%, v/v), extraction temperature (^{o}C), extraction time (minutes), and solvent/material ratio (mL/g). Each experiment was carried out 3 times and the average results were taken. When optimizing experimental factors, one factor was modified, while other factors were maintained at a specified value. Further, the obtained values were used in the Box-Behnken model using Design-Expert software to produce 27 sets of process parameters. Analysis of variance (ANOVA) was conducted to check the fitness of the model and the statistical significance of the regression coefficients. Last, optimal conditions were counted from the final model and verified by an actual experiment attempt. Finally, the optimal conditions calculated from the model are tested by an actual experiment. In this study, Design-Expert 11.0.0 (State-Ease Inc., Minneapolis, MN, USA) was used to generate the experimental designs, a statistical analysis, and a regression model. The second-order equation representing the general function form of one desired outcome for independent variables looks as follows:

$$Y = b_0 + \sum_{j=1}^{k} b_j X_j + \sum_{j=1}^{k} b_{jj} X_j^2 + \sum_{u<j} b_{uj} X_u X_j \quad (2)$$

Where Y is the predicted response; b_0 is the intercept coefficient; b_j is the linear coefficient; b_{jj} is the square coefficient; b_{uj} is the interaction coefficient; X_u and X_j are the coded independent variables, terms of X_uX_j and X_j^2 are the intersection and quadratic terms, respectively.

Data processing: The data are expressed as: Mean ± Standard error of the mean (SEM). Data were processed using MS Excel 2016 software, statistical processing based on a t-test.

3. Result and Discussion

3.1. Single Factor Investigation

Fig. 1 showed the influence of factors on extraction yield and total flavonoid content in the crude extract. In Fig. 1a, when the ethanol concentration increased from 40% to 60%, the extraction efficiency and total flavonoid content increased rapidly from 12.1% to 14.8% and 1.67% to 2.35%, respectively. Continuing to increase the concentration of ethanol solvent to 70%, both extraction efficiency and total flavonoid content reached the highest value of 15.2% and 2.49%, respectively. However, when the ethanol concentration was raised to 80%, the extraction efficiency decreased to 14.1% and the

total flavonoid content decreased to 2.44%. It can be seen that, when the concentration of ethanol increased, the polarity of the solvent would gradually decrease to a level consistent with the polarity of the flavonoid group. As a result, at a suitable ethanol concentration, the extraction efficiency and total flavonoid content would reach the maximum value. The results of the research by Dent *et al.* (2013) also showed that the recovery of polyphenol compounds depends on the type of solvent used, its polarity, and the solubility of polyphenol compounds in the extraction solvent [13]. Therefore, the ethanol concentration was chosen as 70% for the following experiments.

In the next study, the effect of temperature on the extraction process was studied in Fig. 1b. When the extraction temperature increased from 45°C to 65°C, the extraction efficiency and total flavonoid content increased quickly and reached 15.44% and 2.54%, respectively. Continuing to increase the temperature to 75°C and 85°C, the extraction efficiency increased slightly to 15.61% and then decreased to 14.91%; for total flavonoid content, there was a tendency to decrease gradually reaching values of 2.45% and 1.98%, respectively. It can be seen that, when the extraction temperature was increased to a certain value, the extraction efficiency and total flavonoid content would reach the maximum value because at a higher extraction temperature, the surface tension of the solvent decreased, increasing the rate of diffusion and mass transfer allowed faster extraction. However, too high extraction temperature would occur oxidation reactions that reduced flavonoid content. Besides, the additional mucilage and colloidal compounds could be formed in high temperature conditions that reduce extraction efficiency [14]. Therefore, we chose the extraction temperature of 65°C as the base level for further experiments.

Fig. 1c shows the effect of extraction time on extraction efficiency and total flavonoid content. When the extraction time was increased from 60 min to 120 min, both extraction efficiency and total flavonoid content increased linearly from 8.78% to 13.73% and 1.67% to 2.35%, respectively. Continuing to increase the extraction time to 150 minutes, the extraction efficiency increased to 15.22% and the total flavonoid content reached the maximum value of 2.55%. When the extraction time was increased to 180 min, the extraction efficiency changed very little and the total flavonoid content tended to decrease. It can be seen that initially, increasing the extraction time allowed the solutes to better diffuse into the solvent. However, at a certain time, an equilibrium would be established and even increasing extraction time would not produce any perceptible modification in extraction yield [15]. In contrast, flavonoid or polyphenol compounds were less stable to heat and easily degraded when extraction time was prolonged at high temperature, which leads to a decrease in total flavonoid content. These experimental results were in agreement with the results of a previous work in which the extraction procedure of polyphenols from *Glycine max* L. seedlings was investigated [16]. Based on the obtained results, we selected the extraction time of 150 min as the base level for the subsequent experiments.

Finally, the effect of the extraction ratio on extraction yield and total flavonoid content was shown in Fig. 1d. When the solvent/material ratio increased from 5/1 (mL/g) to 20/1 (mL/g), the extraction efficiency and total flavonoid content increased fast from 6.95% to 14.94% and 1.17% to 2.49%, respectively. Continuing to increase the extraction ratio to 30/1 (mL/g), the extraction efficiency and total flavonoid content had a minor increase to 15.22% and 2.52%, respectively. At the extraction ratio of 40/1 (mL/g), the values of these did not change significantly. This could be explained as the increased solvent/material ratio improved the surface contact between the material and the solvent, allowing the compounds to be dissolved more easily. At first, the solute concentration difference between the raw material and the solvent was large, the mass transfer of the solute from the raw material into the solvent took place at a high speed. When equilibrium was reached, diffusion slowed down even though the solvent volume increases [17],[18]. Based on the obtained results, in order to meet the economic efficiency, we chose the extraction ratio of 20/1 (mL/g) as the base level for the experiments.

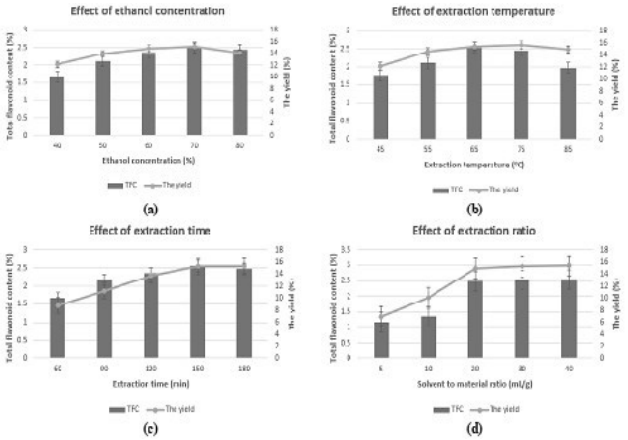

Fig. 1. Effect of ethanol concentration (a), extraction temperature (b), extraction time (c), and extraction ratio (d) on the yield and total flavonoid content from *Passiflora foetida* L.

3.2. Predicted Model and Statistical Analysis

Model setting

Based on the results of the single-factor experiment in section 3.1, we set up the variation range and experimental levels of 4 independent variables including ethanol concentration (%, v/v), extraction temperature (^oC), extraction time (min) and solvent to the material ratio (mL/g) (Table 1). Table 2 included 27 different sets of experimental conditions designed according to the Box-Behnken model. The results of the target functions including extraction yield Y1 (%) and total flavonoid content Y2 (%) were calculated. These values were the basis for evaluating the compatibility of the model with the experiment.

Table 1. Independent variables and their corresponding levels

Independent Variables	Codes	Avariable range (Δ)	Levels		
			-1	0	1
Z1: Ethanol concentration (%, v/v)	A	10	60	70	80
Z2: Extraction temperature (^oC)	B	10	55	65	75
Z3: Extraction time (min)	C	30	120	150	180
Z4: Solvent/material ratio (v/w)	D	10	10	20	30

Table 2. Experimental design and response values

Run	A	B	C	D	Y1-Yield (%)	Y2-TFC (%)
1	-1	-1	0	0	10.59 ± 0.11	1.86 ± 0.07
2	+1	-1	0	0	10.24 ± 0.09	1.91 ± 0.08
3	-1	+1	0	0	8.10 ± 0.12	2.13 ± 0.11
4	+1	+1	0	0	12.44 ± 0.14	2.11 ± 0.13
5	0	0	-1	-1	7.60 ± 0.11	2.07 ± 0.10
6	0	0	+1	-1	8.93 ± 0.17	1.62 ± 0.05
7	0	0	-1	+1	14.08 ± 0.12	2.04 ± 0.13
8	0	0	+1	+1	15.46 ± 0.13	2.09 ± 0.12
9	-1	0	0	-1	8.73 ± 0.07	2.02 ± 0.07
10	+1	0	0	-1	6.23 ± 0.14	2.01 ± 0.16
11	-1	0	0	+1	8.94 ± 0.09	2.21 ± 0.15
12	+1	0	0	+1	16.90 ± 0.17	2.22 ± 0.14
13	0	-1	-1	0	10.03 ± 0.12	1.96 ± 0.09
14	0	+1	-1	0	10.48 ± 0.13	1.94 ± 0.08
15	0	-1	+1	0	13.37 ± 0.11	1.49 ± 0.11
16	0	+1	+1	0	9.36 ± 0.11	1.98 ± 0.12
17	-1	0	-1	0	8.42 ± 0.12	1.88 ± 0.09
18	+1	0	-1	0	10.39 ± 0.19	2.14 ± 0.06
19	-1	0	+1	0	10.62 ± 0.11	2.10 ± 0.17
20	+1	0	+1	0	13.58 ± 0.13	1.90 ± 0.11
21	0	-1	0	-1	9.77 ± 0.11	2.00 ± 0.14
22	0	+1	0	-1	8.75 ± 0.15	1.76 ± 0.12
23	0	-1	0	+1	14.08 ± 0.08	1.71 ± 0.08
24	0	+1	0	+1	15.74 ± 0.14	2.22 ± 0.07
25	0	0	0	0	12.89 ± 0.13	2.36 ± 0.15
26	0	0	0	0	14.33 ± 0.16	2.55 ± 0.16
27	0	0	0	0	13.50 ± 0.12	2.42 ± 0.15

Anova analysis

To analyze the validity of the regression equations Y1 and Y2, the values of F, p, and the coefficient of determination (R^2) were used to evaluate the results of the linear regression analysis. The predicted reaction surfaces for extraction yield (Y1), and total flavonoid content (Y2) were described by independent variables expressed by quadratic polynomial equations such as mathematical equation number (3), (4). The ANOVA analysis of the quadratic regression model demonstrated that the model was significant, and fit sufficiently with the experiment data. The F-values for Y1 and Y2 were calculated as 18.18 and 17.82, respectively, and the very low p-value ($p < 0.0001$) suggested that all models were statistically significant (Table 3). The coefficients of determination (R^2) are 0.9550 and 0.9541, respectively, which means that 95.50% and 95.41% of the response surface variability can be explained by the experimental results. Besides, the lack of fit, which was used to find out the adequacy of the fit, was not significant ($p > 5\%$) for both Y1 and Y2. Based on the above analyzed data, we could conclude that both the objective functions Y1 and Y2 were regressive and compatible with the real tests [19],[20].

Table 3. Regression coefficients of second-degree polynomial models for yield (Y1), and total flavonoid content (Y2)

Source	Y1		Y2	
	F - Value	P - Value	F - Value	P - Value
Model	18.18	< 0.0001*	17.82	< 0.0001*
A	22.44	0.0005*	0.08	0.7735^{NS}
B	1.11	0.3119^{NS}	23.24	0.0004*
C	11.57	0.0053*	11.30	0.0057*
D	134.35	< 0.0001*	15.46	0.0020*
AB	7.18	0.0200*	0.25	0.6228^{NS}
AC	0.32	0.5839^{NS}	9.81	0.0087*
AD	35.65	< 0.0001*	0.01	0.9156^{NS}
BC	6.47	0.0258*	12.27	0.0044*
BD	2.34	0.1522^{NS}	26.38	0.0002*
CD	0.006	0.9803^{NS}	11.28	0.0057*
A^2	30.34	0.0001*	17.55	0.0013*
B^2	8.72	0.0121*	95.54	< 0.0001*
C^2	9.95	0.0083*	85.53	< 0.0001*
D^2	4.85	0.0480*	38.62	< 0.0001*
Lack of Fit	1.56	0.4523^{NS}	0.4894	0.8197^{NS}
R-Squared	0.9550		0.9541	
Adj- R-Squared	0.9024		0.9006	
Adeq Precision	16.9886		16.5698	

* $p<0.05$; NS = not significant

From the experimental data and established mathematical model, 02 regression equations had been determined corresponding to extraction efficiency (Y1) and total flavonoid content (Y2) as follows:

$Y1 = 13.57 + 1.2A + 0.86C + 2.93D + 1.17AB + 2.62AD - 1.11BC - 2.09A^2 - 1.12B^2 - 1.2C^2 - 0.83D^2$ (3)

$Y2 = 2.44 + 0.10B - 0.07C + 0.08D - 0.11AC + 0.13BC + 0.19BD + 0.12CD - 0.13A^2 - 0.31B^2 - 0.29C^2 - 0.19D^2$ (4)

Response Surface Analysis

Based on the experimental 2nd-order polynomial model, the experimental data were analyzed by response surface methodology using Design-Expert 11.0.0 software. The X and Y axes of the three-dimensional response surface represented two extraction factors, the Z axis was one of the two evaluation indexes of extraction efficiency and total flavonoid content. The response surfaces were characterized in Fig. 3.

The response surfaces presented the simultaneous influence of two factors on the value of the objective functions. In Fig. 3a, six response surfaces had been showing the pairs of simultaneous interactions of 2 out of 4 extraction factors on extraction efficiency. The red and yellow areas on the response surfaces were the optimal region, which contained the largest set of values. Based on the response surfaces, the best variable value range of each extraction factor was determined as followed: ethanol concentration from 70-75 (%, v/v), extraction temperature from 60-70^{o}C, extraction time from 150-170 minutes and solvent/material ratio from 20/1 to 30/1 (mL/g). Then the extraction efficiency reached the maximum value in the range of 14 - 16%. In addition, the positive influence of the pairs of factors of ethanol concentration/extraction temperature, ethanol concentration/extraction time, and extraction temperature/extraction time on the objective function Y1 was smaller than those of the remaining pair of extraction factors based on the size of the optimal region in the surface area.

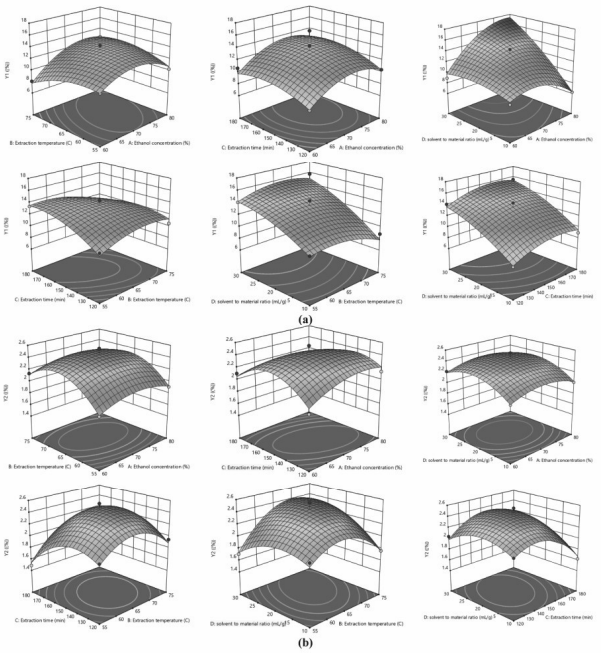

Fig. 3. Response surface of the yield - Y1 (a) and total flavonoid content - Y2 (b)

Similar to Fig. 3a, Fig. 3b included 6 response surfaces showing the simultaneous influence of pairs of extraction factors on total flavonoid content. Based on these response surfaces, the best range of variation values of each factor was as followed: ethanol concentration from 70 - 80 (%, v/v), extraction temperature from 65 - 70^{o}C, extraction time from 150 - 160 minutes, and solvent/material ratio from 20/1 to 25/1 (mL/g). With these conditions, the value of total flavonoid content reached the maximum value in the range of 2.4 - 2.6%. Besides, the positive influence of the pairs of factors of extraction temperature/extraction time, and ethanol concentration/extraction time on the Y2 objective function value was smaller than those of the remaining pair of extraction factors based on the size of the optimal region in the surface area.

Model optimization and verification

The model was optimized with the goal that both functions Y1 and Y2 have the maximum value. The priority of the two selected objective functions included extraction efficiency (Y1) at level 2 and total flavonoid content (Y2) at level 5. The predicted values at the optimal conditions according to the model showed the maximum value of the two objective functions of extraction efficiency and total flavonoid content were 15,2507% and 2,4621%, respectively. At optimal conditions, the values of extraction factors were: ethanol concentration 71.7 (%, v/v), extraction temperature 68.09^{o}C, extraction time 151.18 minutes, and extraction ratio 25.2/1 (mL/g) (Fig. 4).

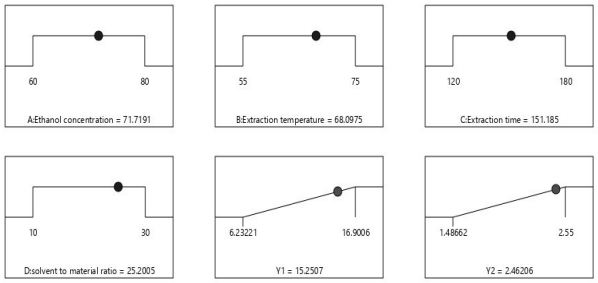

Fig. 4. Optimal conditions by solution of ramps

Table 5. The result for optimal extraction conditions

Optimal conditions				Target functions	Experimental value**	Predicted value
A	B	C	D			
71.71 (%, v/v)	68.09 (^{o}C)	151.18 (min)	25.2/1 (mL/g)	Y_1	14.95 ± 0.16%	15.2507%
				Y_2	2.39 ± 0.09	2.4621%

*** Mean value of three experiments (n = 3).*

After a batch of tripled_experiments with the optimal conditions, the values of the objective functions Y1 and Y2 were obtained as shown in Table 5. The results in Table 5 showed that the model for the total flavonoid extraction from *Passiflora foetida* L. was confirmed when the predicted and experimental values of the objective functions had a difference of less than 5%.

A preliminary experiment showed that the raw sample of *Passiflora foetida* L. had a total flavonoid content of 0.4% (calculated by absolute dry weight). After conducting extraction under optimal conditions, the obtained extract contained a total flavonoid content of 2.39% with a crude extract extraction yield of 15%. The total flavonoid content in the extract was increased by about six times compared with that of the raw material, and the total flavonoid extraction yield was about 90%. In the optimal process, the extraction efficiency is sufficient while ensuring economic factors. These optimal extraction conditions are the essential information for further scale-up of production.

4. Conclusion

Using RSM combined with design expert 11.0.0 software, the model had been developed and optimized the conditions for flavonoid extraction from *Passiflora foetida* L. The results showed that the model is compatible with the experiment. The optimal conditions of four factors were ethanol concentration of 71.71% (v/v), extraction temperature of 68.09^{o}C, extraction time of 151.18 minutes, and extraction ratio of 25.2/1 (mL/g). At optimal conditions, the extraction yield and total flavonoid content were 14.95 ± 0.16% and 2.39 ± 0.09. respectively. The flavonoid-rich extract from *Passiflora foetida* L. was a good material for further scale-up on extraction experiments as well as a great potential for the exploitation and creation of different medicinal forms to serve the needs of human health care.

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LC-MS/MS METHOD DEVELOPMENT FOR DETERMINATION OF ILLEGAL ADDITION OF FIVE H1 ANTIHISTAMINES IN HERBAL PRODUCTS

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Summary

LC-MS/MS Method Development for Determination of Illegal Addition of Five H1 Antihistamines in Herbal Products

A sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the simultaneous analysis of five H1 antihistamine compounds in herbal products. The validation procedure was performed to evaluate selectivity, linearity, LOD, LOQ, accuracy, and precision according to AOAC and ICH guidelines. The linearity of the method estimated using correlation coefficient (R^2) was found to be greater than 0.99 for all compounds in the range of concentration 1-50 ng/ml. The LOQs were 1 ng/ml for all target compounds. The accuracy (expressed as recovery) was 80.2-109.1%. The intraday and interday precision were not more than 10.37% and 9.63%, respectively, at the tested concentration levels. The developed method was applied for the determination of five antihistamines in herbal products collected from markets, websites or received from users. As a result, 6 in 35 samples (17.1%) were adulterated with the target compound at concentration ranging from 0.048 to 3.13 mg/g. The proposed method is suitable for monitoring the adulteration of H1 antihistamine compounds in herbal products.

Keywords: *LC-MS/MS, Method, H1 antihistamine, Adulteration, Herbal product.*

1. Introduction

Demand for dietary supplements that support healthy lifestyles has steadily increased worldwide over the years. Over the past decade, the adulteration of active ingredients and unapproved pharmaceuticals into dietary supplements has increased and poses a global challenge in controlling the safety of natural supplements and natural products. The ease of purchasing across multiple distribution channels coupled with an increasingly complex global supply chain, has led to widespread distribution of illegal herbal preparations and dietary supplements around the world. The abuse of illegal drugs causes side effects and adverse effects, such as heart attack, mental disorder, increased blood pressure, hallucinations, tachycardia, agitation, vomiting, convulsions… [1],[2].

In Vietnam, the demand of herbal preparations in disease treatment and health promotion is very high. However, several active pharmaceutical ingredients such as glucocorticoids, phosphodiesterase inhibitors type 5, hypoglycemia have been detected in different herbal preparations [3]. Aware of this risk, in the Circular 10/2021/TT-BYT of the Ministry of Health has listed 80 abandoned pharmaceutical chemicals and derivatives that are banned in the production and trading of food for