Original Article:

Response Surface Methodology for Optimization of Supercritical Fluid Extraction of Orange Peel Essential Oil

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ABSTRACT

Background: Orange peel essential oils were obtained using supercritical fluid extraction. This method is an important high scaling extraction method used for the extraction of plant and animal extracts.

Objectives: The aim of this study was the optimization of the extraction of the orange peel essential oil.

Methods: The experimental parameters of Supercritical Fluid Extraction (SFE) such as temperature, pressure, and extraction time, and modifier volume were optimized using a central composite design after a 24-1 fractional factorial design. Orange peels were collected from ripe orange and were washed, air dried, and milled. Then, a Suprex MPS/225 system in the SFE mode with a maximal operating pressure of 395 bars was used for essential oil extraction. Moreover, GC-MS and GC-FID were used for the identification and determination of oil compounds, respectively.

Results: Eight compounds have been identified based on their retention indices and mass spectra. According to the results, α-pinene, β-pinene, myrcene, d-limonene, terpinolene, C8-aldehyde, citronellol, and linalool were identified in orange peel essential oil. The optimum SFE conditions were obtained at a pressure of 347.07 atm, temperature of 55° C, extraction time of 30.16 min, and ethanol volume of 147.05 µL. Moreover, extraction yields based on SFE varied in the range of 0.04% to 1.18% (w/w).

Conclusion: The Results showed supercritical fluid technology as the best alternative technique for the extraction of pure and high-quality essential oil from orange peel. It is a green method and does not have any environmental impact.

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Introduction

Various methods are used for the extraction of bioactive compounds from natural sources. In pharmaceutical and nutritional procedures, the extraction procedures must be done with the highest yielding and lowest destructions [1]. The conventional extraction methods such as maceration, percolation, and Soxhlet have some disadvantages, including low yielding, high processing time, non-industrial production, and high probable destruction of compounds. So, novel extraction methods are needed for the semi-industrial and industrial extraction of valuable natural compounds [2, 3].

Supercritical Fluid Extraction (SFE) is an applicable method for higher yielding and continuous extraction of natural compounds. Besides, the extracted compounds are preserved in this method. According to the literature, the SFE method usually was the main method for the extraction of the natural compounds in pharmaceutical industries [4, 5].

The Response Surface Methodology (RSM) is a statistical-mathematical method used for the optimization of effective factors in experimental procedures such as extraction and secondary metabolite production. Moreover, the software calculations of RSM can help to determine the optimized conditions of the extraction of natural pharmaceutical or nutritional compounds [6].

The Rutaceae family is an important flowering plant, which is used for various nutritional and pharmaceutical purposes in the world. This family has about 160 genera and over 1600 species that are usually endemic (or endogenous) in temperate regions. The Citrus genus is the main important genus of this family, which comprises the more applicable species, including *Citrus sinensis* L., Osbeck (Orange), *Citrus limon* L., Osbeck (Lemon), *Citrus aurantium* L. (Bitter orange), and *Citrus reticulate Blanco* (Tangerine). These plants have highly fragrant flowers and fruit peels, which are used in commercial oil production for nutritional and pharmaceutical purposes [7, 8].

Citrus sinensis L. Osbeck or sweet orange is an important plant in the family. Moreover, the essential oils of the flower and fruit peel and the extracts of the fruit of this plant are extensively used as flavoring and sweetening agents for providing and optimization of formulations in nutritional and pharmaceutical industries [9, 10]. As these derivatives are valuable, the high yielding and affordable extraction of the orange essential oils or extracts are needed.

The main aim of this study is the evaluation of the applicable and effective conditions of supercritical fluid extraction of orange peel essential oil as an important ingredient in the nutritional and pharmaceutical industries.

Materials and Methods

Collection method

The plant specimen was collected from Ramsar City, Mazandaran Province, Iran, in November 2015 (36°54′11″N, 50°39′30″E). Also, a plant specialist performed taxonomic identification of plant samples, and representative voucher specimens were deposited in the herbarium of the Active Pharmaceutical Research Center, Pharmaceutical Science Branch, Islamic Azad University, Tehran.

Experimental section

Orange peels were obtained and then dried in a dark place at room temperature. Besides, the cleaned orange peels were milled in a laboratory miller (Myson, China) and passed through a set of standard mesh sieves. Finally, a powder with an approximate particle size of 0.5 mm was collected and stored at 4° C.

HPLC (High Performance Liquid Chromatography) grade ethanol, hexane, and methanol were purchased from Caledon (George Town, Ont., Canada). Moreover, H2SO4 and Na2SO4 were purchased from Merck (Darmstadt, Germany). Carbon dioxide (99.99% purity), contained in a cylinder with an educator tube, was obtained from Roham Co. (Tehran, Iran).

In this study, the Design-Expert software 7.0.0 (Minneapolis, USA) was employed for experimental design and data analysis. In addition, a central composite design after a 24-1 fractional factorial design was used to examine the effects of the variables on extraction yield. The experimental independent variable, including A. Pressure; B. Dynamic extraction time; and C. Modifier volume were used for the optimization of the extraction of essential oils. Table 1 presents the coded levels of independent variables. Moreover, the design included 18 runs, which are shown in Table 2. All experiments were carried out in triplicate. Besides, based on the screening study, the temperature and static extraction time has been set at 55oC for 10 min [11, 12].

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode with a maximal operating pressure of 395 bar was used. Plant powder (0.5 g) was mixed well with 1-mm diameter glass beads and was charged into the 3-mL

Table 1. Coded levels of independent variables of central composite design of the study

Variables	Coded Symbols	Levels				
		-α *	-1	0	+1	+α
Pressure (atm)	A	180.91	215	265	315	349.09
Dynamic Extraction Time (min)	B	28.18	35	45	55	61.82
Modifier Volume (µL)	C	62.96	80	105	130	147.04

*α: 1.682.

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Table 2. Experimental design and response values in the central composite design of the study

Run	A (atm)	B (min)	C (µL)	Total Yield (%)
1	0	0	-1.682	0.32± 0.02
2	0	0	0	0.76± 0.06
3	-1	1	-1	0.08± 0.01
4	1.682	0	0	1.03± 0.13
5	-1.682	0	0	0.15± 0.05
6	0	0	1.682	0.99± 0.04
7	1	-1	-1	0.47± 0.01
8	0	1.682	0	0.59± 0.09
9	-1	-1	1	0.43± 0.02
10	0	0	0	0.75± 0.07
11	-1	1	1	0.46± 0.08
12	-1	-1	-1	0.12± 0.01
13	0	0	0	0.77± 0.04
14	1	1	-1	0.69± 0.03
15	0	-1.682	0	0.30± 0.06
16	0	0	0	0.76± 0.09
17	1	-1	1	0.87± 0.04
18	1	1	1	1.18± 0.10

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stainless steel extraction vessel. The extracted essential oils were collected in ethanol. Besides, for all the modifier studies, the modifier (ethanol) was spiked directly on to the sample in the extraction vessel before the extraction cell was attached to the SFE system. The oil recovery percentage was calculated by weighing the collected solution after the evaporation of ethanol at room temperature [11, 13]. The essential oil that was obtained from the optimized conditions was analyzed using gas chromatography coupled mass spectrometer.

The gas chromatograph was equipped with a 30-m DB-5 (5% biphenyl+95% methyl polysiloxane) fused-silica capillary column, 0.53mm I.D., and 1.5µm film thicknesses (Folsom, CA, USA). Besides, Helium (99.999% purity) was used as carrier gas with a flow rate of 1.2 mL/min. The temperatures of the injector and de-

Table 3. ANOVA results of the study

Source	df	Sum of Squares	Mean Squares	F *	P *
A	1	10.8133	10.8133	10.81	0.0010
B	1	0.8175	0.8175	0.82	0.3659
C	1	5.7922	5.7922	5.79	0.0161
AA	1	0.4699	0.4699	0.47	0.4930
AB	1	0.4005	0.4005	0.40	0.5268
AC	1	0.0378	0.0378	0.04	0.8458
BB	1	1.8619	1.8619	1.86	0.1724
BC	1	0.0253	0.0253	0.03	0.8736
CC	1	0.2345	0.2345	0.23	0.6282
Total error	8	0.0336	0.0042	0.00	1.0000
Total (corr.)	17	19.9673	-	-	-

* Significant

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tector were set at 250° C and 300° C, respectively. Oven temperature program was 100° C for 1 min, increased to 200° C at a rate of 4° C/min held for 2 min, and raised at a rate of 1° C/min to 210° C and held for 2 min, and finally raised at a rate of 4° C/min to 300° C and held for 3 min [14, 15]. Moreover, the structures were determined using a mass spectrometer (HP5970, USA), operating in EI mode at 70eV. The interface temperature was 250° C. Mass range was 30-600 amu [14, 15]. The sample (1 µL) was injected with a split ratio of 1/10. The library searches and spectral matching of the components were conducted on the NIST MS 2.0 database [14].

3. Results

The Box-Cox plot of power transforms was demonstrated in Figure 1. This plot represents the Lambda value +1.12 with a lower confidence interval of +0.91 and a higher confidence interval of +1.33. Moreover, a natural log was used instead of the predicted values. Moreover, Table 3 presents the result of ANOVA. Also, Figure 2 represents the predicted values versus actual values of the study.

The effects of all parameters on essential oil extraction yield were analyzed. The Response Surface of the studied variables was presented in Figure 3. As can be seen, Figure 3A shows the effects of pressure and extraction time on the essential oil extraction; Figure 3B shows the effects of pressure and modifier volume on the essential oil extraction and Figure 3C shows the effects of extraction time and modifier volume on the extraction of essential oil.

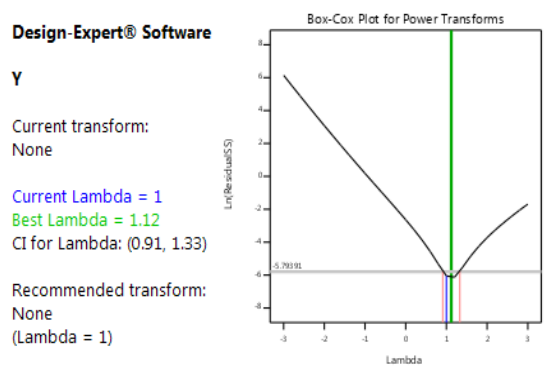

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Figure 1. The Box-cox plot for power transforms

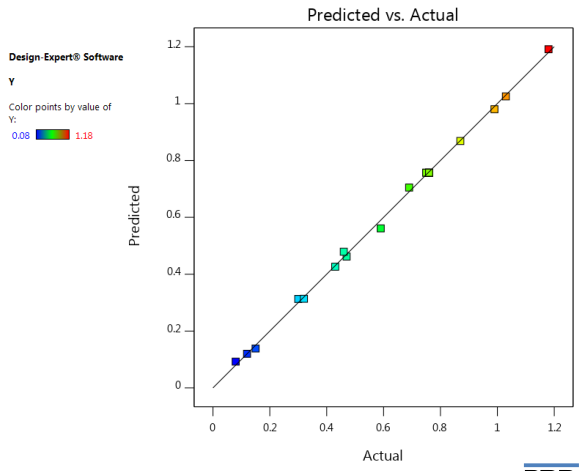

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Figure 2. Actual values versus the predicted values for the extraction of essential oil

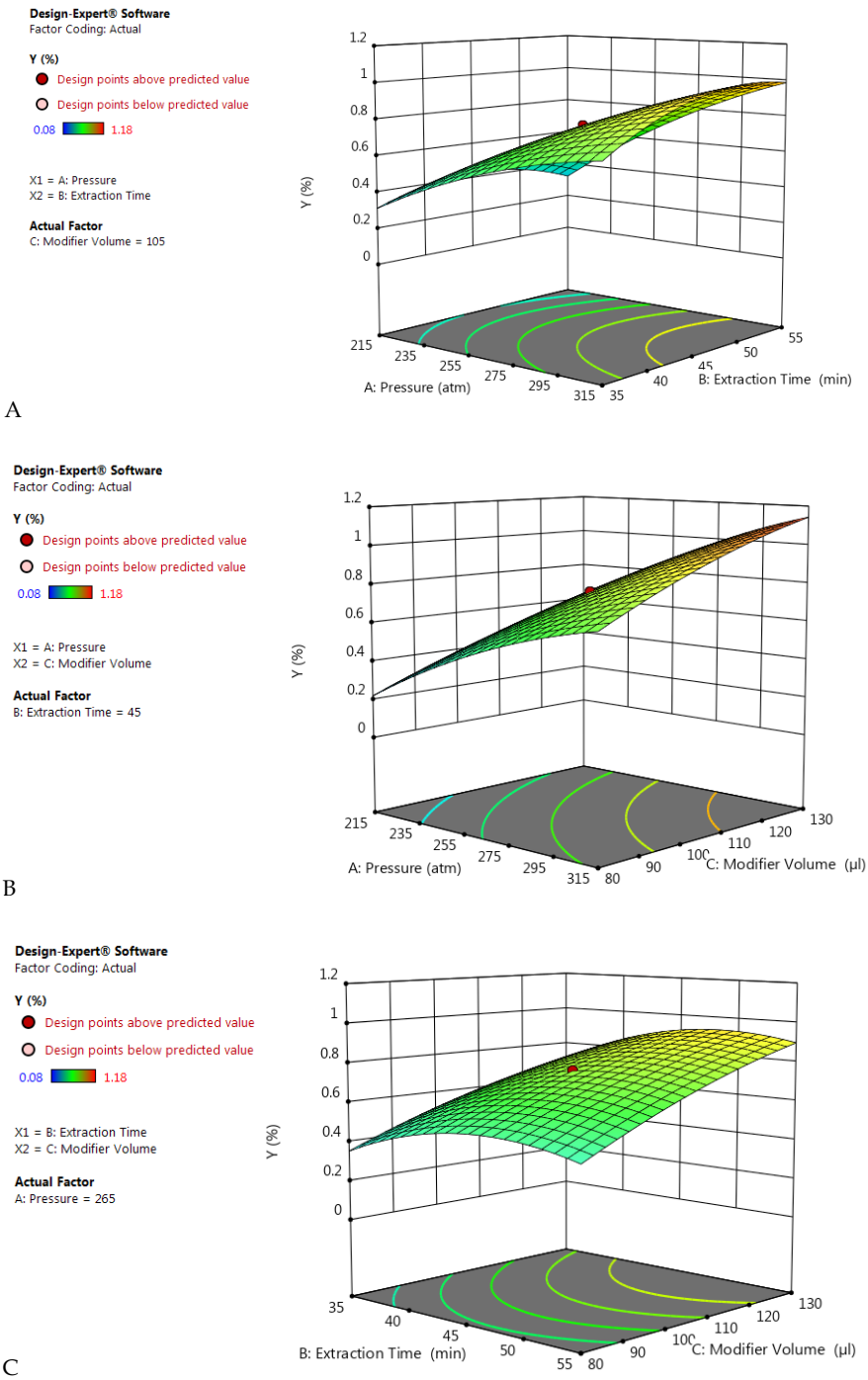

Figure 3. Response surface and contour plots of the study parameters

A: Effects of extraction time and pressure on the response Y (the percentage of essential oil); B: Effects of extraction time and modifier volume on the response Y (the percentage of essential oil); C: Effects of pressure and modifier volume on the response Y (the percentage of essential oil)

In addition, Figure 4 illustrates the effect of the interaction of variables on the extraction yield of essential oils. Figure 4A demonstrates the interaction between the pressure and extraction time. Figure 4B displays the interaction between the pressure and modifier volume. Figure 4C shows the interaction between extraction time and modifier volume.

In this study, the extraction of essential oil was optimized by the supercritical fluid extraction method and statistical-mathematical response-surface methodology. Moreover, three variables, including pressure, extraction time, and modifier volume have been selected and analyzed using the Box-Benhken method and central composite design.

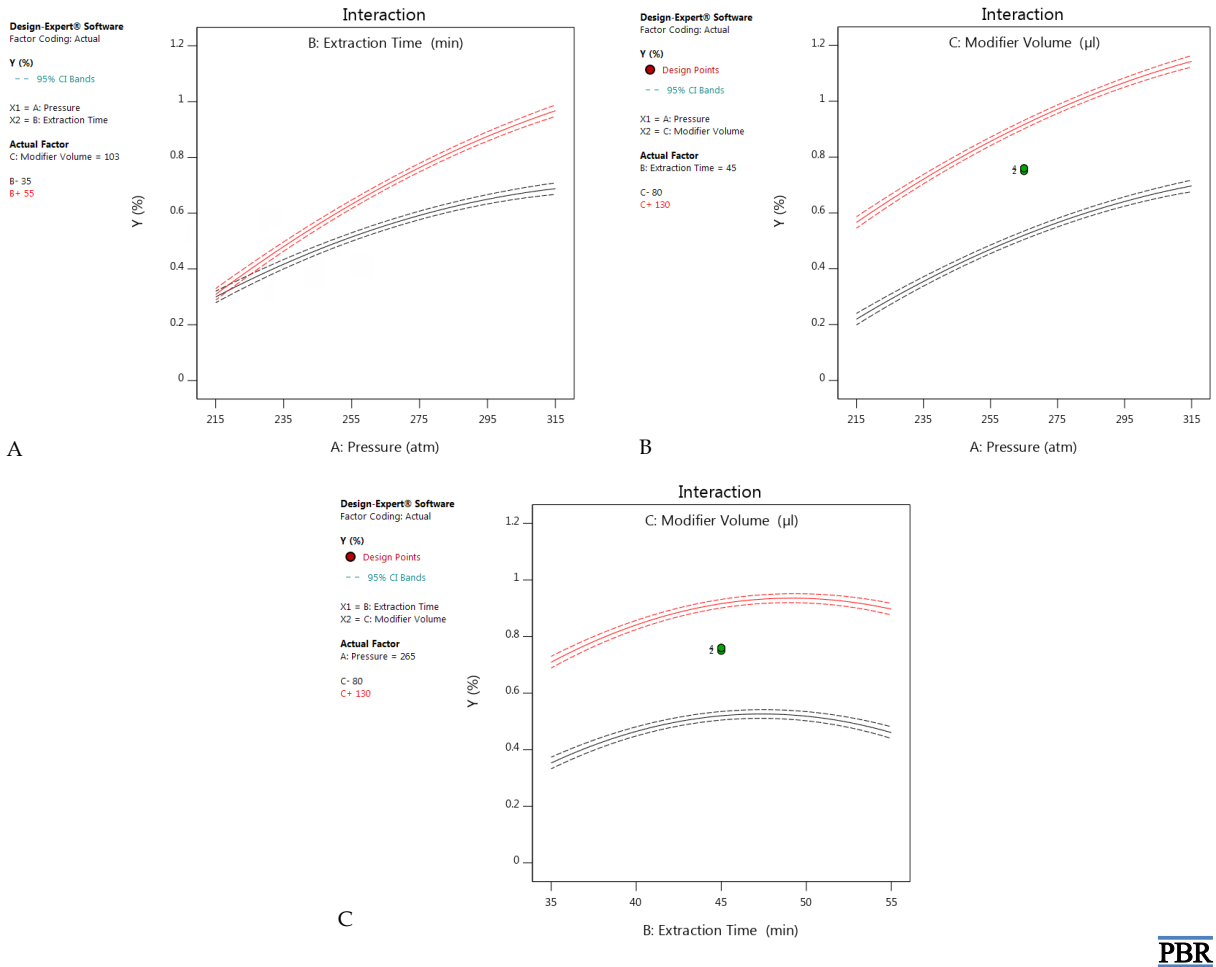

Figure 4. Effect of interaction of parameters on the extraction yield of colchicine

A: Interaction of extraction time and pressure; B: Interaction of extraction time and modifier volume; C: Interaction of pressure and modifier volume

According to this analysis, the Lambda value creates the best power fit model. Moreover, the studied model has a higher F value and a low and significant P value, which is suitable for the optimization of parameters of colchicine extraction. Besides, the coefficients are statistically significant ($P<0.05$). So, this model is suitable

Table 4. Composition of Citrus sinensis (L.) Osbeck essential oil

Compounds	Retention Time (min)	%
α-Pinene	4.56	7.68
β-Pinene	10.24	4.31
Myrcene	15.63	4.22
Terpinolene	25.36	8.25
D-limonene	27.63	58.26
Aldehyde C8	29.54	6.76
Citronellol	34.63	5.75
Linalool	40.80	4.77

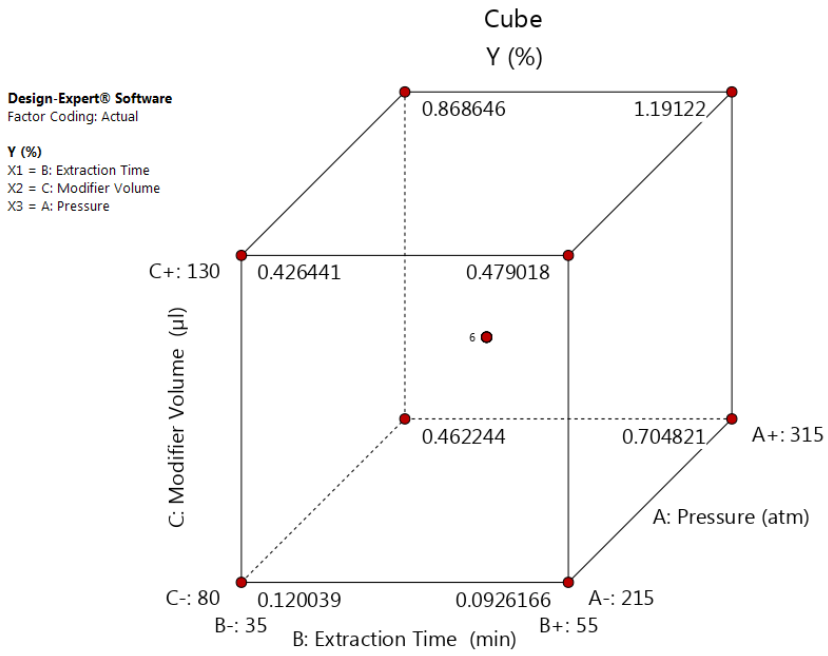

Figure 5. The cubic plot of the effects of three variables on the extraction of essential oils

for the prediction of the essential oil extraction yield within the ranges of variables. In this study, the number of analyses was calculated using the following formula:

N=2P+2P+C, where P refers to the number of variables, and C refers to the number of central points.

Moreover, the obtained equation was presented as:

$$R= -13.9916+0.0339676X^{1}+0.230975X^{2}+0.0493033X^{3} - 0.0000755626X^{12}+0.00035X^{1}X^{2}+0.000082X^{1}X^{3} - 0.00379828X^{22}+0.00043X^{2}X^{3} - 0.000322052X^{32}$$

where R is the extraction yield, X1 refers to the pressure, X^2 denotes the extraction time, and X^3 is the modifier volume.

According to Figures 1 and 2, the study model is fit and the significance level of all results was lower than 0.05. Figures 3, 4, and 5 showed that all variables have direct effects on the yield of extraction of essential oils. Moreover, the pressure was more effective than other variables, and increasing the pressure of the apparatus decreases the extraction time and modifier volume.

Figure 6 and Table 4 present the GC-MS data of obtained essential oil by the optimized condition. According to these data, 8 volatile compounds of α-pinene, β-pinene, myrcene, terpinolene, d-limonene, C8-aldehyde, citronellol, and linalool were detected and assayed. Besides, d-limonene has the highest amount.

Discussion

The optimization studies of the extraction of natural compounds with nutritional and pharmaceutical purposes could be done more efficiently to simply and affordably achieve the nutritional and pharmaceutical products. Many studies were done in the world for the optimization of the extraction of natural compounds by different methods, especially the supercritical fluid extraction method, ultrasound-assisted method, and microwave-assisted method.

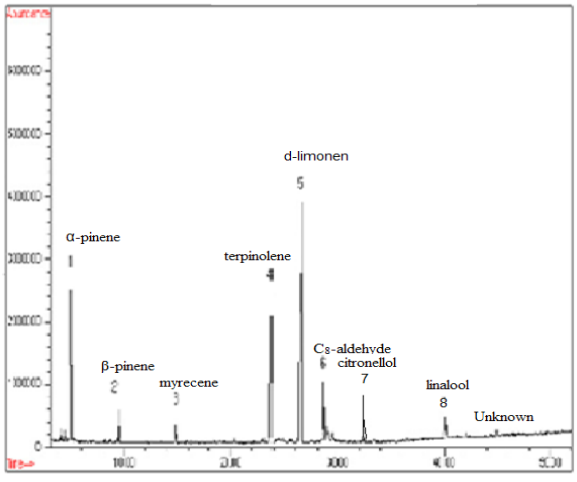

Figure 6. The chromatogram of essential oil obtained by optimized condition

Létisse et al. evaluated the optimization of the extraction of EPA and DHA from sardine by supercritical fluid extraction coupled RSM. According to this study, CO2 rate and extraction time were the main parameters for the optimization of sardine oil extraction [16].

Sodeifian et al. explained the optimized condition of the extraction of omega-3 from *Dracocephalum kotschyi* seed oil by supercritical fluid extraction method. In this study, the CO2 rate, pressure, extraction time, and modifier volume were effective for the extraction [17].

Azadbakht et al. evaluated the optimization of extraction of colchicine from Colchicum *kurdicum* by ultrasonic-assisted extraction method. In this study, temperature, extraction time, and solvent/plant ratio were selected as the variables [18].

Ghafoor et al. studied the optimization of supercritical fluid extraction of the bioactive compounds of *Vitis labrusca* B. by RSM. In this study, the extraction was carried out according to an orthogonal array design and independent selected variables were temperature, pressure, and modifier concentration. In addition, the process was optimized by using RSM for the extract yield, total phenols, antioxidants, and total anthocyanins from the peel [11].

Kassama et al. evaluated the optimization of supercritical fluid extraction of lycopene from tomato skin with a central composite rotatable design model. In this study, the variables were temperature (40° C and 70° C), pressure (25 and 45 MPa), and modifier concentration (5% and 15%). In addition, the highest yield was predicted at 62° C, 45 MPa pressure, and 14% modifier concentration, and the recovery of all trans-lycopene was 33% [19].

SFE is an applicable method for high yielding extraction of natural compounds. This method has many controllable parameters, which helps to extract with less sample and shorter extraction time. Moreover, a central optimized design after a screening step was used to optimize the extraction conditions. In this model, the optimum extraction conditions, including 347.07 atm pressure, 55° C, 30.16 min extraction time, 147.05 µL modifier volume, and the extraction yield of 1.18%.

Ethical Considerations

Compliance with ethical guidelines

This study approved by the ethics committee of Islamic Azad University.

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Authors' contributions

All authors contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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