

Optimization of variables for pressurized fluid extraction of essential oil from *Lavandula angustifolia* using Box-Behnken design

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Abstract: Essential oil components were extracted from *Lavandula angustifolia* (Lavender) flowers using pressurized fluid extraction (PFE). A Box-Behnken design was used to optimize the effective extraction variables. The chemical composition of extracted samples was analyzed by gas chromatograph-flame ionization detector column. For achieving maximum % extraction yield the temperature, pressure, and dynamic extraction time were adjusted 85.1 °C, 36 bar, and 21.8 min, respectively. The PFE is a practical technique for separation of constituents such as 1,8-Cineole (7.25%), linalool (37.15%), linalyl acetate (24.03%), and camphor (8.31%) from *Lavandula angustifolia* to be applied in the food, fragrance, aromatherapy, and pharmaceutical industries. The PF method is quicker (21.8min versus 8 h) and more extraction yield (8.42 versus 4.9) than Soxhlet
Keyword: Pressurized Fluid Extraction; *Lavandula angustifolia*; Lavender; Box-Behnken Design; Soxhlet

1. Introduction

One of the valuable sources of medicines and food is plants. Most of active chemicals gained from these unique bio-resources have remedial and therapeutic properties, including anti-cancer and anti-bacterial activities [1-3]. Due to the high proportion usage of plant extracts in the pharmaceutical and medical industries, it is necessary to conduct theoretical and experimental research to produce the needed extracts. In this regard, the essential oil of lavender showed a broad spectrum antiplatelet effect and was able to inhibit platelet aggregation induced by arachidonic acid, collagen and the stable thromboxane receptor agonist U46619 with no prohemorrhagic properties [4]. Linalyl acetate seemed to be the main active antiplatelet agent. Aroma inhalation of lavender essential oil or one of its main components, linalool, can significantly decrease symptoms associated with tension, anxiety and stress. They may also be applicable to the treatment of menopausal disorders via inhalation [5]. Lavender essential oil demonstrated an interesting analgesic activity, mainly relevant after inhalation, at doses devoid of sedative side effects [6]. Moreover, inhalation of the volatile fractions of lavender (0.1– 0.2 mg/m³ air) reduced the cholesterol content in the aorta and also atherosclerotic plaques [7], but had no effect on the content of cholesterol in the blood. Allergic symptoms can also be suppressed by lavender oil [8]. A lavender fragranced cleansing gel had a significant transient effect of improving mood and making people feel more relaxed [9]. Even foot soaking in warm water containing lavender essential oil followed by reflexology treatment with jojoba oil containing lavender, appeared to be effective for alleviating fatigue in terminally ill cancer patients [10]. The extraction of essential oil from flowers and leaves represents an attempt to isolate the desirable mixture while preserving the original composition with natural properties [11].

Pressurized fluid extraction (PFE, Dionex trade name of accelerated solvent extraction (ASE)) is an efficient, rapid, selective, and reliable extraction method [12-15]. PFE technology and its applications have been developed and used by various scientists [16]. Moreover, it has been applied for the quantitative extraction of different samples of environmental organic compounds from soils and lipids, and also for the analysis of food and biological samples [17]. The possibility of changing several extraction variables such as temperature, pressure, and volume of solvent is a promising characteristic of available automated PFE instruments [18]. Using a modified supercritical fluid extraction (SFE) apparatus, we have developed a new PFE method, mainly as an analytical tool, to determine oil content or as a sample preparation method for the oil quality measurements with full capability of automation when the workload is high [19]. Special emphasis is placed on obtaining a rapid, selective, efficient, and reliable extraction process. A few studies were performed in regard to separation of essential oil from *Lavandula angustifolia*. Essential oil was extracted from Italian *Lavandula angustifolia* [20] using supercritical carbon dioxide and compared with hydrodistillation and sonication [21]. A new process design and operation for microwave accelerated steam distillation of essential oils from Italian *Lavandula angustifolia* was developed and compared with steam distillation [22].

The aim of this study is extraction of the essential oil from *Lavandula angustifolia* (Lavender) flowers using pressurized fluid extraction method for the extraction of the major compounds of this plant including 1,8-cineole, linalool, linalyl acetate, and camphor. A Box-Behnken (B-B) design [23] was used to guide experimentation the experiment and a mathematical model was constructed to investigate the relationship between the extraction yield and the variables influencing extraction such as temperature, pressure, and dynamic extraction time. The validity of the model was evaluated through the analysis of variance (ANOVA) technique by using Minitab software [24]. The essential oil obtained by Soxhlet was used for comparison. The further investigations are being performed in our research laboratory for using this technique for the large-scale extraction as a multipurpose extraction method.

2. Materials and methods

2.1 Materials

Lavender flowers samples were obtained from Isfahan Agricultural Research Center. Ethanol (99.6%, Merck) was utilized as the solvent for pressurized fluid extraction. N-hexanol (99.6%, Merck) was utilized as the internal standard for the GC-FID calibration analysis. Pure 1,8-Cineole (470826, $\geq 99\%$, Aldrich), linalool (51782, $\geq 99\%$, Fluka), linalyl acetate (45980, $\geq 95\%$, Fluka), and camphor (148075, 96%, Aldrich) were used as the standards as the four important components of the Lavender essential oil. Industrial grade carbon dioxide ($\geq 99\%$, Zamzam) was used as the supercritical fluid.

2.2 Preparation of Lavender flower

The Lavender flower was dried at 40 °C for a period of 3 hr prior to extraction. In the end of the normal drying process of Lavender the water residue was around 10.2%. Following the extraction procedures flowers were finely grinded using laboratory equipments [25]. Since extraction kinetics in this study was controlled by the kernel particle size, an important sieving step was carried out to achieve reproducible extraction yield in which the samples were passed through a sieve with mesh sizes between 20 and 30 (particle diameters ranging over 0.60-0.85 mm). The dried samples were kept within sealed bag in the cold and dry place until they were used.

2.3. Soxhlet extraction

Traditional Soxhlet extraction was carried out in standard apparatus by standard method [26]. In this method extraction was done for 8 hours with 50 mL of ethanol solution (n-hexanol as an internal standard was added to ethanol with concentration 2500 ppm before extraction).

2.4. PFE procedure

The equipment used for PFE is very similar to that used in supercritical fluid extraction. Since CO₂ must be liquefied by using cooler circulator device prior to its pumping, It would be easier to operate with ethanol than CO₂. Most published studies described a similar arrangement for the PFE equipments [27-29]. For this research, the apparatus was modified using a switching valve after the pumps to enable pumping of the liquid solvent and CO₂, alternatively into the extraction vessel, and the back pressure regulator [24, 27]. The apparatus used for PFE is shown in Figure 1. The prepared Lavender flower sample (~30g) was loaded into the 100 mL cylindrical stainless steel cell. Based on the commonly used PFE and SFE methods Cotton wool was packed at the exit end of the cell to prevent transfer of solid samples to the tubing and clogging of the system [16]. The PFE method was performed dynamically by passing ethanol with constant flow rate of 0.5 mL/min at temperatures, pressures, and dynamic times through the extraction cell. The ethanol residue in the cell, tubing, and back pressure regulator was removed with purging the PFE system with CO₂ at the end of each extraction. To reach the mentioned goal, firstly the CO₂ should be converted into the supercritical state, and after that it will dissolve any contamination remaining in PFE system. Ethanol was pumped into the system to wash the tubing, every time the system tubing was clogged [16].

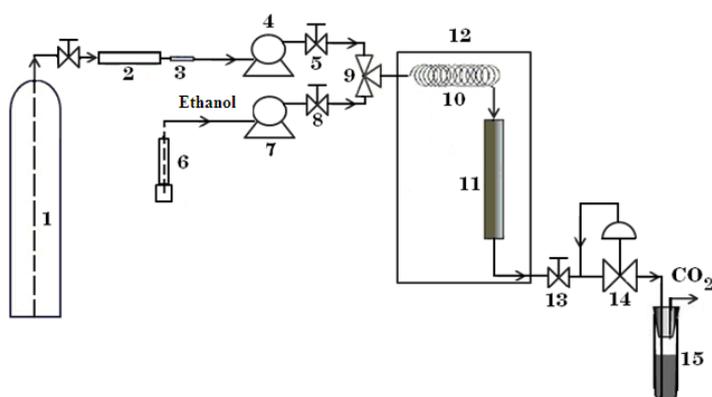


Figure 1. Schematic diagram of PFE system: (1) CO₂ tank; (2) molecular sieve filter; (3) ss 2 μm pore size filter (4) carbon dioxide transfer pump; (5, 8, 13) two-way needle valves; (6) Ethanol tank; (7) high-pressure piston pump; (9) three ways valve; (10) preheating coil; (11) extraction cell; (12) thermostated oven; (14) back-pressure regulator; (15) sample collection vessel.

2.5. GC-FID analysis

Four compounds were separated and determined using gas chromatography (GC). A gas chromatograph (Agilent Technologies, Model 6890N) was used with Helium (He) (purity >99.99%) as the carrier gas, a HP-5 capillary column (30 m long, 0.25 mm I.D. and 0.25 μm film thick), and a flame ionization detector (FID). A sample injection volume of 0.2 μL in each analysis and the internal standard method was used to obtain the highest possible precision for quantitative GC measurements. The injection port and the detector temperatures were 230 °C and 250 °C, respectively. Temperature programming was also used to separate the extracted components as follows: the initial oven temperature was 60 °C for 1 min which was then increased to 120 °C at a rate of 8 °C/min where it was kept for 2min to be subsequently increased to 220 °C at a rate of 20 °C/min. It was finally kept at 220 °C for 1min before terminating the program. The amounts of 1,8-Cineole, linalool, linalyl acetate, and camphor quantified by calculating the area under the chromatographic peaks divided by the area of n-hexanol as an internal standard (A_s/A_{is}). In order to obtain the calibration curves, several solutions with different concentrations of 1,8-Cineole, linalool, linalyl acetate, and camphor in ethanol were injected into the GC-FID and the area under each peak was calculated, and the results were precisely obtained. The four linear calibration curves were fitted using a linear regression line with $R^2 \geq 0.98$. Finally, using the calibration curves, the extraction yield (Y) was determined using Eq. (1).

$$Y = (\text{total mass of four components in extracted sample} / \text{mass of dried Lavender flower}) \times 100 \quad (1)$$

2.6. Experimental design

A statistical experimental design based on “Box-Behnken (B-B)” was planned [17] and the extraction yield were measured for different variables such as temperature, pressure, and dynamic extraction time coded as x_1 , x_2 , and x_3 , respectively. These variables were investigated at three levels (-1, 0, 1) and the dependent variables were Y . We used the Minitab software package [16] to design and evaluate these three independent variables at three levels on the responses according to the Eq. (2). The ranges for the selected levels of the three variables are shown in table 1. The experimental extraction yield for different selected levels of variables is shown in table 2 for 15 runs.

$$Y = \beta_0 + \sum \beta_j \cdot X_i + \sum \beta_{ij} \cdot X_j^2 + \sum \beta_{jk} \cdot X_j \cdot X_k \quad (2)$$

Where, Y = response and X_i , X_j^2 , X_j , X_k = level of independent variables.

Moreover the theoretically predicted values of yield at different experimental conditions are illustrated in table 2.

Table 1. Range of values for the response surface methodology

| Independent variables | Levels | | |
|-----------------------|--------|----|-----|
| | -1 | 0 | 1 |
| Temperature (°C) | 60 | 80 | 100 |
| Pressure (bar) | 10 | 30 | 50 |
| Dynamic time (min) | 5 | 20 | 35 |

Table 2. Yield for the different selected levels of variables

| Run | Temperature (°C) | Pressure (bar) | Dynamic time (min) | Observed Yield (w/w %) | Predicted Yield (w/w %) |
|-----|------------------|----------------|--------------------|------------------------|-------------------------|
| 1 | 80 | 30 | 5 | 5.926 | 6.23863 |
| 2 | 80 | 10 | 20 | 5.462 | 5.36808 |
| 3 | 100 | 30 | 35 | 6.659 | 7.01989 |
| 4 | 80 | 50 | 35 | 7.115 | 6.84275 |
| 5 | 80 | 30 | 5 | 6.032 | 6.23863 |
| 6 | 60 | 50 | 20 | 4.584 | 5.18738 |
| 7 | 60 | 30 | 5 | 5.280 | 4.31361 |
| 8 | 100 | 30 | 20 | 7.495 | 7.34611 |
| 9 | 60 | 10 | 5 | 1.316 | 1.70950 |
| 10 | 100 | 50 | 5 | 5.515 | 5.51394 |
| 11 | 80 | 30 | 5 | 5.973 | 6.23863 |
| 12 | 80 | 50 | 20 | 8.049 | 7.71892 |
| 13 | 80 | 10 | 35 | 5.088 | 4.99936 |
| 14 | 60 | 30 | 20 | 5.838 | 5.80750 |
| 15 | 100 | 10 | 5 | 2.246 | 2.03506 |

3. Result and discussion

3.1. PFE optimum conditions

For maximum % PFE yield, temperature, pressure, and dynamic time were 85.1 °C, 36.0 bar, and 21.8 min, respectively. The optimum condition might provide the design basis for an industrial-scale extraction process. A second-order polynomial equation is proposed for the prediction of PFE yield as a function of different variables as follows:

$$Y = -23.3733 + 0.5762 T + 0.2364 P + 0.1998 t_d - 0.0036 T^2 - 0.0037 P^2 - 0.0054 t_d^2 \quad (4)$$

Where, T is extraction temperature, P is extraction pressure, and t_d is dynamic extraction time. The response surface model which was obtained from an experimental design was evaluated using ANOVA and analysis of residuals. The results of the statistical analyses including the t-test, and p-

values of the extraction yield were tabulated in table 3. The linear regression coefficients, R^2 for the PFE yield was also 95.36 as shown in Figure 2, which shows good performance of the model based on the observed and predicted yields.

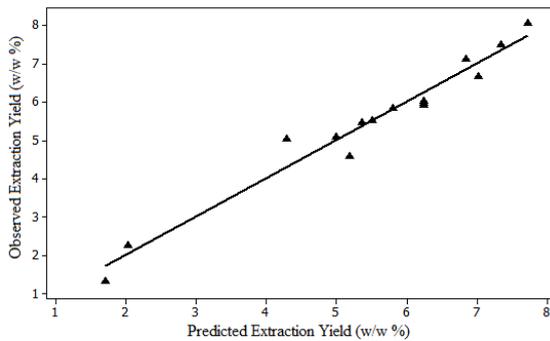


Figure 2. Observed extraction yield versus predicted extraction yield.

Table 3. The t-value, and p-values for the model estimated using Minitab software.

| Term | Extraction Yield | |
|--|------------------|---------|
| | t-value | p-value |
| Constant | - 4.036 | 0.010 |
| T (°C) | 4.073 | 0.010 |
| P (bar) | 2.632 | 0.046 |
| t _d (min) | 1.424 | 0.214 |
| T ² (°C) ² | - 4.119 | 0.009 |
| P ² (bar) ² | - 3.408 | 0.019 |
| t _d ² (min) ² | - 2.936 | 0.032 |
| T (°C)×P(bar) | 0.783 | 0.469 |
| T (°C)×t _d (min) | 0.583 | 0.585 |
| P (bar)×t _d (min) | -0.751 | 0.486 |

The value of significance of each coefficient determined by t-test and p-values are listed in table 3. The larger the t-value and the smaller the p-value, the more significant is the corresponding coefficient. Based on the statistical results (ANOVA) with confidence level of 95%, the effect of each term in the models could be significant provided that its p-value be smaller than 0.05 (p-value<0.05). The results of the extraction yield in table 3 indicate that the interaction terms have weak effect on the extraction yield. It is imperative to realize that even though p-value>0.05 (table 3) for the linear term of dynamic time but due to Hierarchy rule in which the p-value<0.05 for the higher order (quadratic) of this variable, therefore, the effect of all of the linear terms must be considered in the model.

3.2. Variables affecting PFE

3.2.1. The effect of extraction temperature

Increasing the temperature of the extraction increased the % extraction yield as shown in Figures 3 (response surface) and 4 (contour plots) for the extraction yield. An extraction temperature of 85.1°C was sufficient to give maximum extraction yield (8.42%). The increase in yield with temperature is due to increase in ethanol solvating power at higher temperatures [30]. Temperature is the main parameter influencing the physicochemical properties of ethanol and the compounds to be extracted, and it has a great influence on the extraction rate, efficiency, and selectivity in PFE. Enhancement of the extraction efficiency may be related to the increased vapor pressures and accelerated thermal desorption of the compounds from the sample matrix [27]. But the decreases in yield with increasing temperature after a certain temperature (85.1°C) because of the essential oil are degraded above the 85.1 °C.

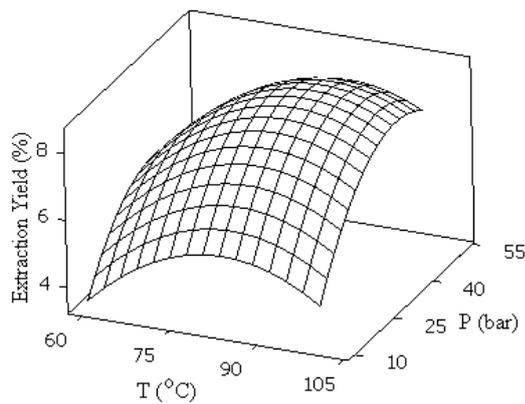


Figure 3. Response surface of the % extraction yield versus T and P at $t_d=21.8$ min.

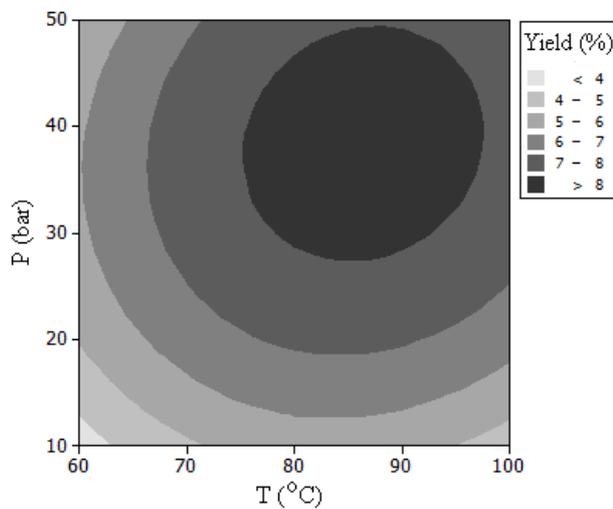


Figure 4. Contour plot of the % extraction yield versus T and P at $t_d = 21.8$ min.

3.2.2. The effect of extraction pressure

Increasing the extraction pressure from 10 to 36 bar increased the extraction yield, while further increases up to 50 bar did not cause any further change as shown in Figure 3. By increasing pressure, interaction between solvent and matrix and the solvent strength is increased up to pressure of 36 bar [31]. In practice, the pressure is kept high enough to maintain the ethanol in liquid form at all extraction temperatures. The presence of pressure could facilitate extraction from samples where analytes are trapped in the matrix pores. This pressure forces the ethanol into areas of the matrices which are not normally covered if ethanol at atmospheric pressure is used [32].

3.2.3. The effect of dynamic extraction time

The PFE was performed at the different static time ranging from 5 to 30 min, but no improvements on the extraction yield were observed. Therefore, the static time was not considered among the parameters; as a result, the effect of dynamic time was studied. Using dynamic extraction, the equilibrium is displaced forward as fresh solvent is continuously pumped through the sample. The extraction time strongly depends on the extraction temperature and the matrix of the analytes, these results showed that increasing temperature decreases the extraction time [33]. Increasing the extraction time, increased the extraction yield up to 21.8 min. Since, the solvent is passing through the extractor continuously, the yield is increased and then a plateau is reached up to 35 min as shown in Figure 5 (response surface) and 6 (contour plots).

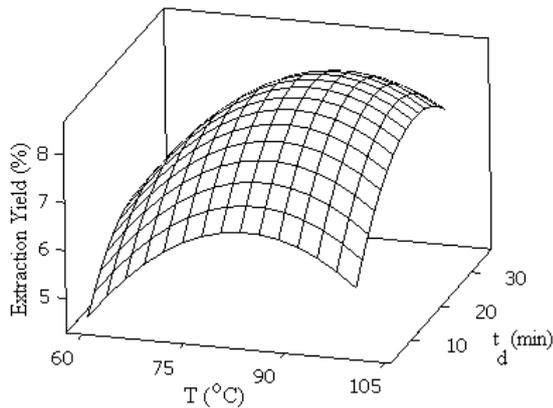


Figure 5. Response surface of the % extraction yield versus T and t_d at P = 36 bar.

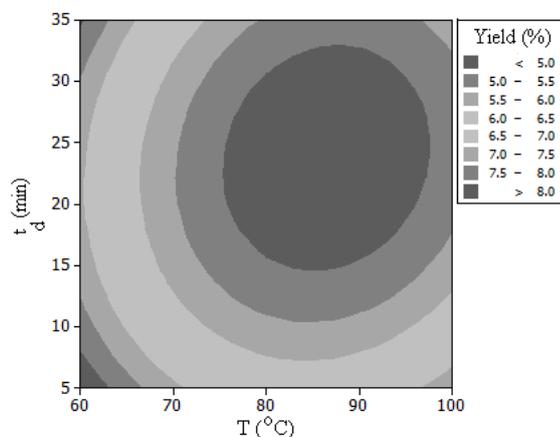


Figure 6. Contour plot of the % extraction yield versus T and t_d at P = 36 bar.

3.3. Comparison of PFE and Soxhlet

Pressurized fluid extraction as well as Soxhlet for the extraction of essential oil from *Lavandula angustifolia*, are compared in terms of time, quality of the essential oil, efficiency and costs. One of the greatest advantages of the PFE method is rapidity. An extraction time of 21.8 min provides comparable yield to those obtained after 8 h of Soxhlet. The essential oils of *Lavandula angustifolia* flowers isolated either by PFE or Soxhlet are rather similar in their composition as shown in table 4. Moreover the *Lavandula angustifolia* flowers essential oil composition of this study is slightly different from to other researches [9, 20, 22]. The ultimate yield of essential oil obtained from *Lavandula angustifolia* flower was 8.42 % by PFE and 4.9 % by Soxhlet: this means the PFE method is 1.72 times more efficient than Soxhlet. These results mean a substantial saving of time, energy and plant material by PFE.

Table 4: Chemical composition of *Lavandula angustifolia* essential oil by PFE and Soxhlet

| method | Components | | | | |
|---------|-------------|----------|-----------------|---------|---------|
| | 1,8-Cineole | Linalool | Linalyl acetate | Camphor | Waxes |
| PFE | 7.25% | 37.15% | 24.03% | 8.31% | 23.26 % |
| Soxhlet | 7.53% | 36.76% | 23.53% | 7.94% | 24.24% |

4. Conclusion

Lavandula angustifolia flowers sample were obtained from Isfahan in Iran to be applied in food, fragrance, aromatherapy, and pharmaceutical industries. In this study, the essential oil was extracted from *Lavandula angustifolia* via pressurized fluid. To achieve maximum extraction yield (8.42 %), the conditions of temperature, pressure, and dynamic time were adjusted 85.1 °C, 36 bar, and 21.8 min, respectively. Furthermore, the Box-Behenken design technique was used to optimize the operating condition variables. The

proposed method consisting on semicontinuous extraction with pressurized fluid extraction is quicker than Soxhlet, provides the quality of the essential oil are rather similar to Soxhlet and allows substantial savings of both energy and investment cost. Its high precision makes it a good alternative for the extraction of essential oils from aromatic plants.

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