Optimization of Process variables for Essential oil from *Lavandula angustifolia* by PHWE using central composite design

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Abstract: Pressurized hot water extraction (PHWE) and hydrodistillation were compared for the extraction of essential oil from Lavandula *angusifolia*. Using GC-FID analysis in conjunction with central composite design, the maximum extraction yield was obtained for temperature of 162.76 ^{o}C, pressure of 29.84 (bar), static time of 10.45 (min), dynamic time of 18.12 (min), and flow rate of 0.69 (mL/min). The PHWE is a practical technique for separation of constituents such as 1,8-Cineole (7.35%), linalool (38.16%), linalyl acetate (17.02%), and camphor (8.23%) from Lavandula *angusifolia* to be applied in the food, fragrance, aromatherapy, and pharmaceutical industries. The PHWE method is quicker (28.57 min versus 3 h) and more extraction yield (7.06 versus 1.03) than hydrodistillation.
Keywords: Optimization; supercritical process Lavandula *angusifolia* flowers;PHWE; Essential oil; Central composite design.

1. Introduction

Essential oils are currently being extracted from natural products either by hydrodistillation or solvent extraction. Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods. These shortcomings have led to the consideration of the use of supercritical fluids in essential oil extraction process. Carbon dioxide is the most commonly used supercritical fluid because of its modest critical condition[1-3]. The green house effect caused by the emission of carbon dioxide, the cost of the fluid with the required purity and specially its low dielectric constant (thus giving rise to a non-polar character that hinders or makes difficult the extraction of polar compounds) make mandatory the searching for new solvents[4]. Above the critical temperature (373.98^{o}C) and critical pressure (224.8 bar) water is in the supercritical state and is an excellent solvent for all kinds of organic compounds. All gases are miscible with supercritical water and to some extent even wood can be dissolved. Unfortunately, supercritical water provides a very reactive environment, where oxidation, hydrolysis and decomposition of compounds can take place[5]. In many cases the quantitative extraction of organic compounds (even non-polar) with water can be made at temperatures lower than the critical temperature and is then called 'subcritical water extraction'[6] or 'Pressurized hot water extraction (PHWE)'[7]. Usually, extraction temperatures lower than 200°C can be used for more polar and less stable compounds and in this way a very selective class extraction of organic compounds according to

their polarity are possible by simply adjusting the temperature[8]. The physical advantages such as high diffusion, low viscosity and low surface tension are achieved at elevated temperature condition. The increased vapor pressures and rapid thermal desorption of target compounds from matrices could enhance the extraction efficiency of PHWE[9]. The high temperatures have also changed the properties of water and thus making the polarity of water closer to those of non-polar compounds[10]. This will enhance the solubility of less polar compounds in water for extraction from different matrices[11].

The supercritical fluid extractor was modified to pump water and CO_2 alternately into the extraction vessel[3]. Using this modified SFE apparatus, we developed a PHWE method as a technique to extraction essential oil from Lavandula *angustifolia* for separation of major compounds of this plants including 1,8-Cineole, linalool, linalyl acetate, and camphor. Lavandula *angustifolia* essential oil to be applied in the fragrance industry (soaps, colognes, perfumes, skin lotion and other cosmetics), in aromatherapy (relaxant), in pharmaceutical preparations for its therapeutic effects as a sedative, spasmolytic, antiviral and antibacterial agent[2], Recently it has also been employed in food manufacturing as natural flavouring for beverages, ice cream, candy, baked goods and chewing gum. A few studies were performed in regard to separation of essential oil from Lavandula angustifolia. Essential oil was extracted from Italian Lavandula angustifolia[13] using supercritical carbon dioxide and compared with hydrodistillation and soniction[14]. 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[15]. Recently we have extracted essential oil from Lavandula angustifolia[16] utilizing supercritical fluid extraction.

We used PHWE and central composite design (CCD)[17] to obtain the optimum conditions for semi-continuous extraction. Variables such as temperature, pressure, static time, dynamic time, and flow rate were investigated. The essential oil obtained by hydrodistillation was used for comparison. To the best of our knowledge, the PHWE method proposed in this study has been used for the first time to extract essential oil from Lavandula *angusifolia* flower.

2. Materials and Methods

2.1 Materials

Lavandula *angusifolia* flowers samples were obtained from Isfahan Agricultural Research Center. Stock standard solution of 3400 μg/mL of n-nonane (74250, analytical standard, Fluka) in HPLC grade hexane (650552, CHROMASOLV® Plus, for HPLC, ≥95%, Sigma-Aldrich) was prepared. Sodium chloride (S7653, BioXtra, ≥99.5%, Sigma-Aldrich) and sodium sulphate (239313, ACS reagent, ≥99.0%, anhydrous, granular, Sigma-Aldrich) were used as demulsifier and drying agent, respectively. N-hexanol (99.6%, Merck) was utilized as the internal standard for the GC-FID calibration analysis. Pure 1,8-Cineole (470826, ≥99%, Aldrich), linalool (51782, ≥99%, Fluka), linalyl acetate (45980, ≥95%, Fluka), and camphor (148075, 96%, Aldrich) were used as the standards as the four important components of the Lavandula *angusifolia* essential oil. Industrial grade carbon dioxide (≥99%, Zamzam) was used as the supercritical fluid.

2.2 Preparation of Lavandula *angusifolia* flower

The Lavandula *angusifolia* flower was dried at 40 °C for a period of 3 hr prior to extraction. In the end of the normal drying process of Lavandula angustifolia the water residue was around 10.2%. Following the extraction procedures flowers were finely grinded using laboratory equipments[18]. 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. Hydrodistillation

The plant (40 g of dried material) was submitted to hydrodistillation for 3 h, using a Clevenger-type apparatus, according to the *European Pharmacopoeia* (1975). The volatile distillate was collected over anhydrous sodium sulphate and refrigerated prior to analysis. Essential oil with 1:100 hexane dilutions

prior to GC-FID analysis was required in order to compare the chromatograms of the essential oil obtained by PHWE with that from hydrodistillation[1,4].

2.4. PHWE Procedure

The equipment used for PHWE is very similar to that used in SFE. Since CO_2 must be liquefied by using cooler circulator device prior to its pumping, It would be easier to operate with water than CO_2. Sample collection is also easier in the PHWE method compared to SFE, as the former deals with liquid solvents at room temperature rather than expanded gas in the latter. However, careful degassing of water is recommended to reduce the amounts of dissolved oxygen, which may otherwise corrode the lines at the high temperatures, used[19]. Most published works describe a similar setup for the PHWE equipments[8,10,19,20]. In dynamic-mode extractions, high-pressure pumps must be efficient enough to pressurize the water and pass it through the sample. Various heating systems such as gas chromatography (GC) ovens, sand baths, or resistive heating blocks have been used to heat and to maintain the extraction vessel at the desired temperature[19]. For the purposes of the present study, the apparatus was modified using a switching valve placed downstream the pumps to enable alternate pumping of the liquid solvent and CO_2 into the extraction vessel[3,21]. The apparatus used for PHWE is shown in Fig. 1. The prepared Lavandula angusifolia flower sample (~4g) and glass bead[22] (broken Pyrex laboratory glassware) with mesh sizes between 20 and 30 (particle diameters ranging over 0.60-0.85 mm) in a ratio of 40–60% (w/w) were loaded into the 10 mL cylindrical stainless steel cell. Based on the commonly used PHWE and SFE[21] 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[19]. The PHWE method was performed dynamically by passing water at different temperatures, pressures, static time, dynamic time, and flow rates. The water residue in the cell, tubing, and back pressure regulator was removed with purging the PHWE system with CO_2 at the end of each extraction to avoid any loss and memory effect[22].

5 ml of hexane and 0.1 mL of n-nonane stock solution were added to 5 mL of each extract in a separating funnel and about 1 g of Sodium chloride was added to facilitate the breaking of the emulsion. The hexane layer was then separated and dried with 0.1 g of anhydrous sodium sulphate before GC-FID analysis[4].
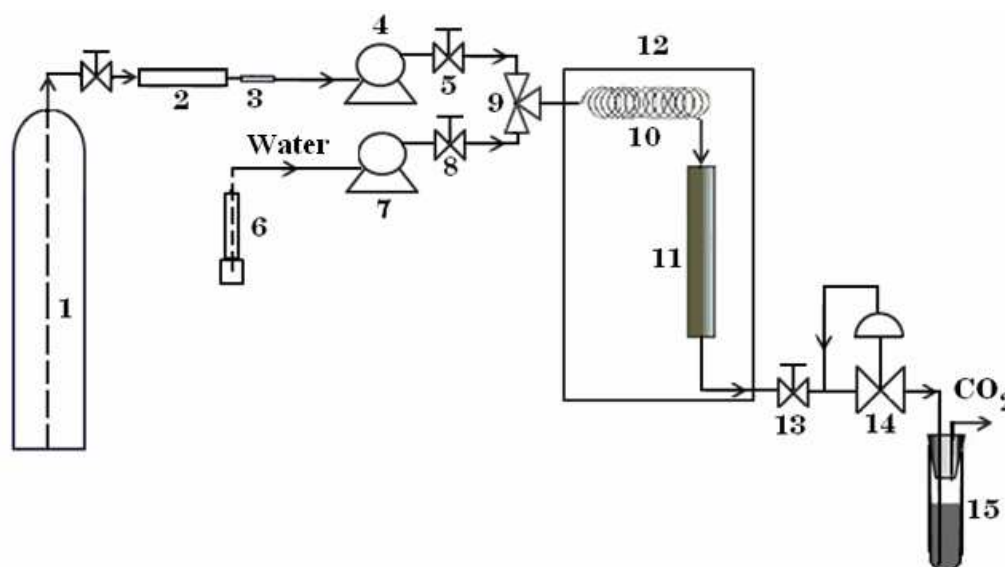

Figure 1. Schematic diagram of PHWE system: (1) CO_2 tank; (2) molecular sieve filter; (3) ss 2 µm pore size filter (4) carbon dioxide transfer pump; (5, 8, 13) two-way needle valves; (6) water tank; (7) high-pressure piston pump; (9) three ways valve; (10) preheating coil; (11) extraction cell; (12) 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) 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 0.2µL 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 $230^{o}C$ and $250\ ^{o}C$, respectively. Temperature programming was also used to separate the extracted components as follows: the initial oven temperature was $60\ ^{o}C$ for 1 min which was then increased to $120\ ^{o}C$ at a rate of 8 $^{o}C/min$ where it was kept for 2 min to be subsequently increased to $220\ ^{o}C$ at a rate of 20 $^{o}C/min$. It was finally kept at $220\ ^{o}C$ 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 (2300 ppm) 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 hexane 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 $R^2 \geq 0.98$, the results of which are plotted in Fig. 2. Finally, using the calibration curves, the extraction yield (Y) was determined using Eq. (1).

Y= (total mass of four components in extracted sample/mass of dried Lavandula angusifolia flower) ×100 (1)
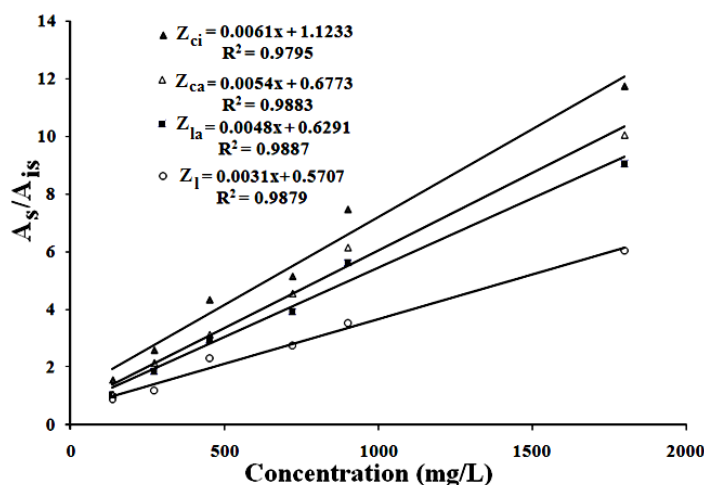
Figure 2. Calibration curves of the pure standards of 1,8-Cineole, camphor, linalyl acetate, and linalool.

2.6. Experimental design

A statistical experimental design based on "central composite design (CCD)" was planned[17] and the extraction yield were measured for different variables such as temperature, pressure, static time, dynamic time, and flow rate coded as x_1, x_2, x_3, x_4, and, x_5 respectively. These variables were investigated at five levels (-2, -1, 0, 1, 2) and the dependent variables were Y. We used the Minitab software package to design and evaluate these five independent variables at five levels on the responses according to the Eq. (3). The ranges for the selected levels of the five variables are shown in Table 1. The experimental extraction yield for different selected levels of variables is shown in Table 2 for 32 runs.

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

Where, Y = response, β_0 = intercept, β_j = linear coefficients, β_{jj} = squared coefficients, β_{jk} = interaction coefficients, 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 central composite half design

Independent variables	Levels				
	$-\alpha^*$	-1	0	1	$+\alpha$
Temperature (^{o}C)	100	125	150	175	200
Pressure (bar)	5	15	25	35	45
Static time (mL/min)	0	5	10	15	20
Dynamic time (min)	5	10	15	20	25
Flow rate (mL/min)	0.2	0.4	0.6	0.8	1.0

*α = star point @ CCD = ± 2

Table 2: Yield for the different selected levels of variables

Run	Temperature (^{o}C)	Pressure (bar)	Static time (min)	Dynamic time (min)	Flow rate (mL/min)	Observed Yield (w/w %)	Predicted Yield (w/w %)
1	125	15	5	10	0.8	1.104	1.25291
2	175	15	5	10	0.4	2.128	2.22424
3	125	35	5	10	0.4	0.264	0.33024
4	175	35	5	10	0.8	4.264	4.29424
5	125	15	15	10	0.4	0.208	0.38491
6	175	15	15	10	0.8	3.656	3.79691
7	125	35	15	10	0.8	2.128	2.23891
8	175	35	15	10	0.4	2.944	3.00242
9	125	15	5	20	0.4	1.488	1.72824
10	175	15	5	20	0.8	4.928	5.13224
11	125	35	5	20	0.8	3.464	3.63824
12	175	35	5	20	0.4	3.992	4.11358
13	125	15	15	20	0.8	2.944	3.22891
14	175	15	15	20	0.4	4.040	4.27224
15	125	35	15	20	0.4	2.248	2.45024
16	175	35	15	20	0.8	7.008	7.17424
17	100	25	10	15	0.6	0.168	-0.22748
18	200	25	10	15	0.6	4.680	4.46185
19	150	5	10	15	0.6	4.816	4.36052
20	150	45	10	15	0.6	5.824	5.66585
21	150	25	0	15	0.6	4.208	3.97385
22	150	25	20	15	0.6	5.312	4.93252
23	150	25	10	5	0.6	1.792	1.68452
24	150	25	10	25	0.6	5.744	5.23785
25	150	25	10	15	0.2	1.984	1.69385
26	150	25	10	15	1.0	5.080	4.75652
27	150	25	10	15	0.6	5.632	5.95427
28	150	25	10	15	0.6	6.240	5.95427
29	150	25	10	15	0.6	6.072	5.95427
30	150	25	10	15	0.6	5.728	5.95427
31	150	25	10	15	0.6	5.888	5.95427
32	150	25	10	15	0.6	5.552	5.95427

3. Result and Discussion

3.1. PHWE optimum conditions

For maximum % PHWE yield (7.06 %), temperature, pressure, static time, dynamic time, and flow rate were 162.76 ^{o}C, 29.84 bar, 10.45 min, 18.12 min, and 0.69 mL/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 PHWE yield as a function of different variables as follows:

$$Y = 5.91441 + 1.27508\,T - 0.56923\,P + 0.34692\,t_s + 0.96408\,t_d + 1.01392\,f - 0.95927\,T^2 - 0.52936\,P^2 - 0.37527\,t_s^2 - 0.62327\,t_d^2 - 0.68227\,f^2 \tag{3}$$

Where, T is extraction temperature, P is extraction pressure, t_s is static time, t_d is dynamic extraction time, and f is flow rate. 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 estimated regression coefficients, t-test, and p-values of the extraction yield were tabulated in Table 3.

The R^2 adjusted of the extraction yield was 95.37. This means that the developed models have been able to fully predict the extraction yield. The linear regression coefficients, R^2 for the PHWE yield was also 98.36 which shows good performance of the model based on the observed and predicted yields.

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 terms in linear forms and in quadratic forms have strong influence on the extraction yield, and the interaction terms have no effect on the extraction yield. It is imperative to realize that even though p-value>0.05 (Table 3) for the linear terms of P but due to Hierarchy rule in which the p-value<0.05 for the higher order (quadratic) of this variable, therefore, the effect of linear terms must be considered in the model.

Table 3: Regression coefficients, t-test, and significant p-values for the model estimated using Minitab software.

Term	Extraction Yield		
	Regression coefficients	*t*-value	*p*-value
Constant	5.91441	26.861	0.000
T (°C)	1.27508	7.074	0.000
P (bar)	-0.56923	-1.523	0.156
t_s (min)	0.34692	1.925	0.081
t_d (min)	0.96408	5.349	0.000
f (mL/min)	1.01392	5.625	0.000
T^2 (°C)^2	-0.95927	-12.307	0.000
P^2 (bar)^2	-0.52936	-3.018	0.012
t_s^2 (min)^2	-0.37527	-4.814	0.001
t_d^2 (min)^2	-0.62327	-7.996	0.000
f^2 (mL/min)^2	-0.68227	-8.753	0.000
T (°C) × P (bar)	0.10275	0.649	0.530
T (°C) × t_s (min)	0.07050	0.668	0.518
T (°C) × t_d (min)	0.03350	0.317	0.757
T (°C) × f (mL/min)	0.08250	0.782	0.451
P (bar) × t_s (min)	0.10725	0.677	0.512
P (bar) × t_d (min)	0.07575	0.478	0.642
P (bar) × f (mL/min)	0.24825	1.568	0.145
t_d (min) × t_s (min)	0.07450	0.706	0.495
f (mL/min) × t_s (min)	0.02550	0.242	0.814
t_d (min) × f (mL/min)	0.06050	0.573	0.578

3.2. Variables affecting PHWE

3.2.1. The effect of extraction temperature

Increasing the temperature of the extraction increased the extraction yield as shown in Fig. 3 for the extraction yield. An extraction temperature of 162.76 ^{o}C was sufficient to give maximum extraction yield (7.06%). The increase in yield with temperature is due to increase in water solvating power at higher temperatures[10]. Temperature is the main parameter influencing the physicochemical properties (viscosity, dielectric constant, and surface tension are reduced at higher temperature) of water and the compounds to be extracted, and it has a great influence on the extraction rate, and efficiency in PHWE. Enhancement of the extraction efficiency may be related to the increased vapor pressures and accelerated thermal desorption of the compounds from the sample matrix[11]. At higher temperature, the strong solute–matrix interaction in the plant materials caused by van der Waals forces, hydrogen bonding and dipole attractions between solute molecules and active sites on the matrix were disrupted[23].
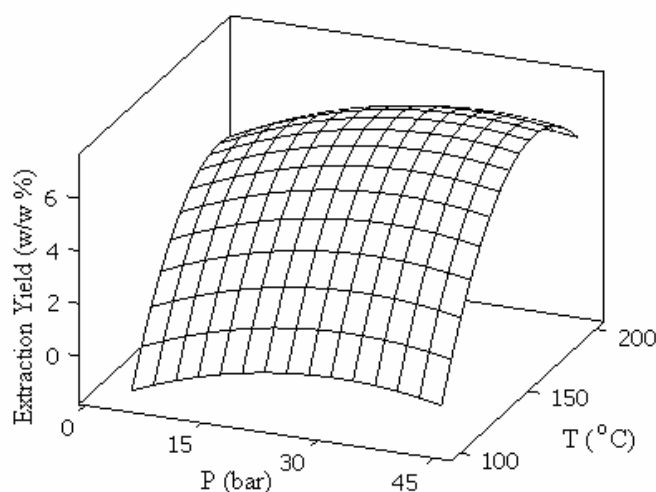
Figure 3. Response surface of the % extraction yield versus T and P at t_s = 10.45 min, t_d= 18.12 min, f = 0.69 mL/min.

3.2.2. The effect of extraction pressure

Increasing the extraction pressure from 5 to 29.84 bar increased the extraction yield, while further increases up to 45 bar did not cause any further change as shown in Fig. 3. By increasing pressure, interaction between solvent and matrix and the solvent strength is increased up to pressure of 29.84 bar[24]. In practice, the pressure is kept high enough to maintain the water 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 water into areas of the matrices which are not normally covered if water at atmospheric pressure is used[8].

3.2.3. The effect of static and dynamic extraction time

In static extraction mode, extraction efficiencies strongly depend on the partition-equilibrium constant and the solubility of compounds. This may cause problems, especially with highly concentrated samples and/or low-solubility analytes. Using dynamic extraction, the equilibrium is displaced to completeness as fresh solvent is continuously pumped through the sample. However, the establishment of a static extraction step before dynamic extraction shortened the time required for complete extraction. It was reported that a 10–20 min static contact time prior to dynamic operation improved the pressurized fluid extraction recovery[20]. Thus in this study, samples were held in the static extraction mode in the range of 0–20 min, followed by a dynamic extraction in the range of 5–25 min. The results of this study indicated that static extraction longer than 10.45 min did not increase extraction yield due to the disappearance of mass transfer driving force as shown in Fig. 4. Increasing

the dynamic extraction time, increased the extraction yield up to 18.12 min. A dynamic extraction time of 18.12 min was sufficient to give maximum extraction yield (7.06%) as shown in Fig. 4.
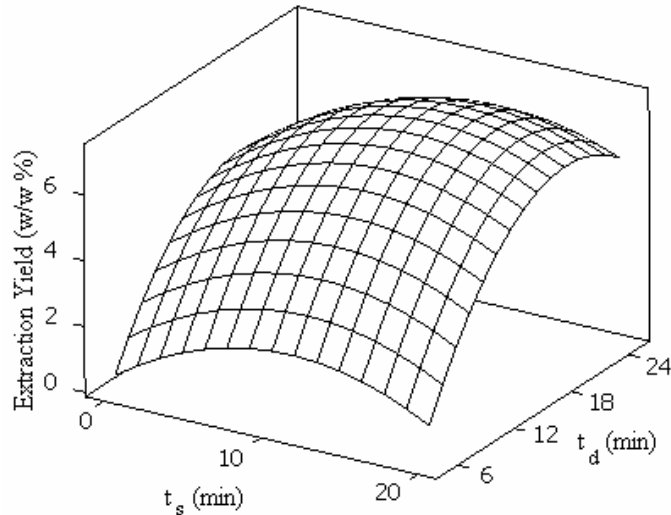
Figure 4. Response surface of the % extraction yield versus t_s and t_d at T = 162.76 ^{o}C, P= 29.84 bar, f = 0.69 mL/min.

3.2.4. The effect of flow rate

Increasing the flow rate increased the extraction yield. Water might be saturated at lower flow rates and the extraction yield reduces, as shown in Fig. 5. An extraction flow rate of 0.69 mL/min was sufficient enough to achieve maximum extraction yield, while further increases up to 1.0 mL/min resulted in little change on extraction yield, at result, we found that the best result was obtained at 0.69 mL/min[10].
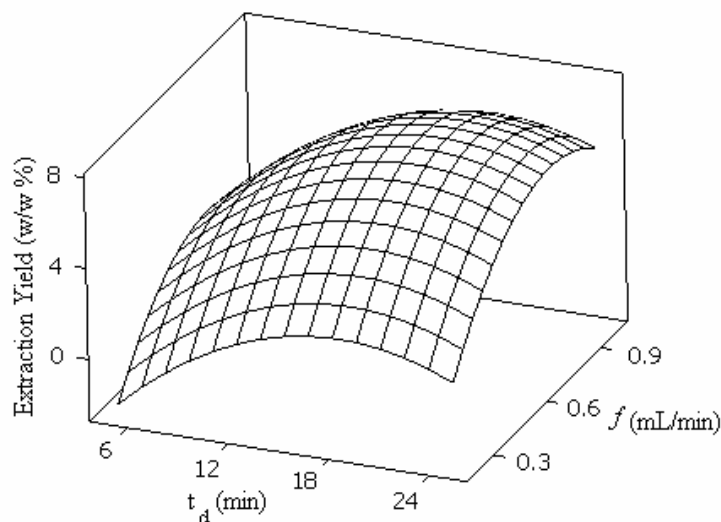
Figure 5. Response surface of the % extraction yield versus t_d and f at T = 162.76 ^{o}C, P= 29.84 bar, t_s = 10.45 min.

3.3. Comparison of PHWE and hydrodistillation

Pressurized hot water extraction as well as hydrodistillation for the extraction of essential oil from Lavandula angusifolia, are compared in terms of time, quality of the essential oil, efficiency and costs. One of the greatest advantages of the PHWE method is rapidity. An extraction time (static + dynamic time) of 28.57 min provides comparable yield to those obtained after 3 h of hydrodistillation. The essential oils of Lavandula angusifolia flowers isolated either by PHWE or hydrodistillation are rather similar in their composition as shown in table 4. Moreover the Lavandula angusifolia flowers essential oil composition of this study is slightly different from to other researches[13-16]. The ultimate yield of essential oil obtained from Lavandula angusifolia flower was 7.064 % by PHWE and 1.03 % by hydrodistillation: this means the PHWE method is 6.86 times more efficient than hydrodistillation. These results mean a substantial saving of time, energy and plant material by PHWE.

Table 4: Chemical composition of Lavander essential oil by PHWE and hydrodistillation

method	Components				
	1,8-cineole	Linalool	Linalyl acetate	Camphor	Waxes
PHWE	7.35%	38.16%	17.02%	8.23%	29.24 %
Hydrodistillation	7.45%	36.17%	18.08%	8.65%	29.65%

4. Conclusion

Lavandula angusifolia flowers sample were obtained from Isfahan in Iran to be applied in food, fragrance, aromatherapy, and pharmaceutical industries. The pharmaceutical and food applications have led to the need of developing better methods of extraction and purification with decreasing utilization of toxic organic solvents. Therefore, in this study, the essential oil was extracted from Lavandula angusifolia via pressurized hot water. To achieve maximum extraction yield (7.06 %), the conditions of temperature, pressure, static time, dynamic time, and flow rate were adjusted 162.76 $^{\circ}$C, 29.84 bar, 10.45 min, 18.12 min, and 0.69 mL/min, respectively. Furthermore, the central composite design technique was used to optimize the operating condition variables. The proposed method consisting on semi-continuous extraction with pressurized hot water extraction is quicker than hydrodistillation, provides the quality of the essential oil are rather similar to hydrodistillation 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.

Notation

Y	Extraction yield
ci	1,8-cineole
ca	Camphor
l	Linalool
la	Linalyl acetate
T ($^{\circ}$C)	Temperature
P (bar)	Pressure
t_s (min)	Static extraction time
t_d (min)	Dynamic extraction time
f (mL/min)	Flow rate
A_s/A_{is}	Peak areas for the sample/ Peak areas for the internal standard

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