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Optimization of supercritical extraction of *Pimpinella affinis Ledeb.* using response surface methodology

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ABSTRACT

In this article response surface methodology (RSM) based on central composite design (CCD) was applied to obtain the optimum conditions and significant parameters in supercritical fluid extraction (SFE) from *Pimpinella affinis Ledeb*. The extracts were analyzed by GC-FID and GC/MS. The major constituent detected in the extracts was trans-α-bergamotene that is an aromatic compound. The effect of operational parameters such as temperature, pressure, bed porosity and mean particle diameter of dried plant were investigated in this process by a statistical approach. Temperature, pressure and mean particle diameter were identified as significant parameters effecting on extraction yield but bed void fraction had no significant effect. Also, the pressure showed as the most effective parameter on the yield of the process. The results indicated that the optimum conditions could be obtained at temperature of 50.72 °C, pressure of 24.39 MPa, particle diameters of 0.36 mm and bed porosity of 0.4.

1. Introduction

Extraction of different compounds from plants can be obtained by the number of processes such as mechanical pressing and grinding, maceration, solvent extraction and distillation. These methods have distinct drawbacks such as time-consuming and labor intensive operations, handling of large volumes of hazardous solvents. High temperatures and water can cause chemical modifications of components. Steam distillation usually results loss of the highly volatile components and some water soluble constituents [1].

Supercritical fluid extraction (SFE) is an alternative to liquid extraction using solvents. The properties of supercritical fluids also provide some advantages for analytical extractions. Supercritical fluids can have solvating powers similar to organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension [2,3]. Due to the low heat of process and the relatively unreactive solvent used in the extraction, the fragrant compounds derived often closely resemble the original odor of the raw material. Like solvent extraction, the SFE takes place at a low temperature, extracts a wide range of compounds, and leaves the aromatics unaltered by heat, rendering an essence more faithful to the original [4].

Since the validity of an experiment is directly affected by its construction and execution, attention to experimental design is extremely important. Response surface methodology (RSM) is a powerful and efficient mathematical approach widely applied in the optimization of process conditions. In statistics, a central composite design (CCD) is an experimental design, useful in RSM, in which a multi-level factorial or fractional factorial is chosen so as to allow the estimation of all first-order and factor interaction terms, augmented with further points, which allow polynomial effects to also be estimated [5–7].

The purpose of this work was to apply CCD based RSM to analyze the effects of the process parameters on extraction yield of extraction from *Pimpinella affinis Ledeb.* by supercritical CO_2 (SC-CO_2) and to search for the optimal values for attaining a higher yield.

Pimpinella has about 150 species in the world which mostly are yearlings. *Pimpinella affinis Ledeb.* is a biennial aromatic plant, 20–110 cm in height, with white umble inflorescences and ellipsoid fruits. It grows wild in the center and north of Iran and is more abundant in Chalous and Khojir [8]. Askari et al. show that the major compound in the hydrodistillation extracted volatile compound from stems/leaves of this plant was identified as *trans-α-bergamotene*, which can be used as an aromatic compound in flavor and fragrance industries [9].

In our previous work, we have reported the results of the effects of pressure and temperature on the yield of extraction [10]. Based on preliminary studies, four variables, such as pressure,

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temperature, mean particle size of the dried plant and bed porosity were considered as influential variables. These variables were examined in the set of experiments based on CCD experimental design and optimal condition for maximum extraction efficiency was obtained. The central composite faced-center (CCF) design with a quadratic model was employed. So, each independent variable had three levels, which were −1, 0 and +1 (based on coded values). A total 17 different combinations (including 3 replicates of center point each sighed the coded value 0) were chosen in random order according to a small-CCD configuration for four factors.

2. Experimental

2.1. Plant material

The aerial parts of *Pimpinella affinis Ledeb*. were collected from Natural Resources Research Station, Nowshahr (a city of Mazandaran Province in Iran) in June/July 2010. After sending to the laboratory, fresh plants were dried at room temperature. The stems/leaves were identified and milled to small particles.

2.2. Reagents

HPLC grade dichloromethane was purchased from Merck Co. to be used as the collecting agent of oil and extracts. CO_2 with purity higher than 99.9% was purchased from Parsa Gas Co. to use as the extracting solvent in supercritical extraction.

2.3. Hydrodistillation

The oils of the plant (30 g) were obtained by hydrodistillation for 3.5–4 h on a Clevenger-type apparatus. The volatile distillate was collected over ether. The amount of oil recovered was 1.2% (W/W), based on the dry weight.

2.4. SFE method

A schematic diagram of the experimental apparatus is shown in Fig. 5. The extraction vessel was approximately 34 ml (stainless steel, height 0.11 m and internal diameter 0.02 m) placed in an equipped oven to regulate the temperature accurately. The powdered plant material (6.0 g) was mixed with glass beads and charged into the extraction vessel. This procedure was used to maximize the contact surface between the sample and the supercritical solvent and to prevent the channeling in the extraction cell. Extraction process was carried out for 30 min in static mode and then 20 min in dynamic mode. The extracted materials collected in a test tube containing 5.0 ml dichloromethane. In order to improve the collection efficiency, the tube was placed in an ice bath during the dynamic extraction stage. Finally, the yield of extracted oil (Y) was calculated from the following equation (Fig. 1):

$$Y = \left(\frac{\text{mass of extract}}{\text{mass of dried plant}}\right) \times 100 \quad (1)$$

2.5. GC and GC/MS analysis

The GC-FID analysis was performed using a Thermo-UFM gas chromatograph with, Ph-5 column (10 m × 0.1 mm i.d., film thickness 0.4 μm), carrier gas; helium at a rate of 0.5 ml min^{-1}. Oven temperature was programmed at 60 °C for 3 min, then increased to 210 °C at a rate of 3 °C min^{-1} and finally increased to 285 °C at a rate of 80 °C min^{-1}. Injector and detector temperatures were kept at 280 °C. GC–MS analyses were carried out on a Varian 3400 GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed at 50–280 °C with rate of 4 °C min^{-1}. Injection chamber temperature was set 10° higher than the final column temperature; carrier gas, helium at a linear velocity of 31.5 cm s^{-1}; split ratio, 1:60; ionization energy, 70 eV; scan time, 1 s; mass range, 40–300 amu.

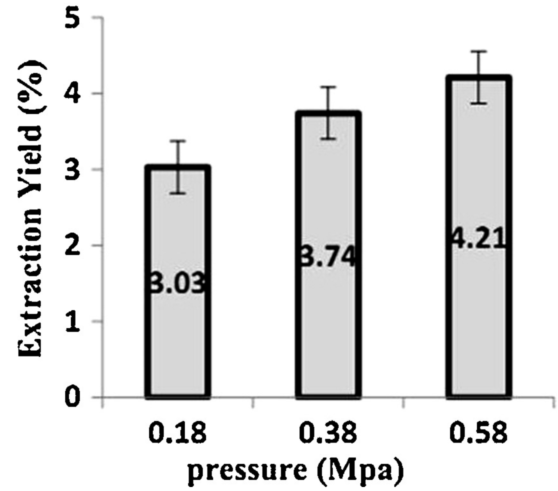

Fig. 1. Schematic diagram of SFE apparatus.

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [17,18].

2.6. Experimental design

A four-factors, three-level central composite design was used to determine the optimal factors of SFE of oil from *Pimpinella affinis Ledeb*. Four independent variables namely temperature (x_1), pressure (x_2), mean particle diameter (x_3) and bed porosity (x_4) was chosen. The coded values of independent variables were found from flowing equations (Table 1):

$$x_1 = \frac{X_1 - 50}{5}$$

$$x_2 = \frac{X_2 - 20}{5}$$

$$x_3 = \frac{X_3 - 0.38}{0.2}$$

$$x_4 = \frac{X_4 - 0.4}{0.1}$$

Table 1
Values of factors in DOE (uncoded and coded).

Factors	Low level (−1)	Center point (0)	High level (+1)
Temperature (°C), X_1	45	50	55
Pressure (MPa), X_2	15	20	25
Mean particle diameter (mm), X_3	0.18	0.38	0.58
Bed porosity (v/v), X_4	0.30	0.40	0.50