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APPLICATION OF MATHEMATICAL MODEL OF NAIK FOR DETERMINATION OF TOTAL EXTRACT YIELD OF HYSSOP OBTAINED BY SUPERCRITICAL EXTRACTION WITH CARBON DIOXIDE

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Abstract*:* In this paper, supercritical extraction of hyssop (*Hyssopus officinalis* L.) is performed by using carbon dioxide as extractant. Effect of pressure (80, 100 and 150 bar) on the yield of total extract at a temperature 313 K, flow 0.00323 kg/min and the average diameter of particles 0.49 mm is investigated. For modeling of extraction system hyssop – supercritical CO_2, Naik's model is applied, where in the total yield of extract is determined. On the basis of the value of the correlation coefficient |r| (0.976 – 0.992), it was concluded that a very strong correlation was obtained between reciprocal values of the total extract yield and extraction time (1/y i 1/t), so it can be concluded that Naik's model can succesfully be applied for determining yield of total extract.
Keywords*:* hyssop, supercritical extraction, modeling, carbon dioxide.

1. INTRODUCTION

Hyssopus officinalis L., belonging to Lamiaceae family and commonly known as 'hyssop', is a polymorphous species that grows as a subshrub on dry, rocky, calcareous soils in Europe, southwestern and central Asia and north-western India. It is cultivated in the USA and former Soviet Union. This species is morphologically and genetically complex, with high variability between populations growing in different areas. So far, several subspecies have been recorded especially for Europe and northern Africa [1].

Hyssop has been exploited for many uses. It is well known for its aromatic scent, and as an ornamental and bee attracting plant [2]. The aerial parts are used in the food industry as a condiment and spice or as a minty flavor [3]. In traditional medicine, the plant has long been used as a carminative, tonic, antiseptic, expectorant and cough reliever. It was also valued in treatment of rheumatic pains, bruises, wounds, and states of anxiety and hysteria, in blood pressure regulation, and has a muscle relaxant [4, 5].

In particular, the essential oil of hyssop is used in liqueurs and cosmetic products and in phytotherapy, though caution is called for, as it contains epileptogenic pinocamphone and isopinocamphone [6]. The oil is also an ingredient of many brands of colognes and perfumes and is used in flavouring alcoholic beverages, meat products, and seasonings [7−9]. The chemical composition of hyssop oil is now specified worldwide by the ISO 9841 (2007).

Due to its considerable economic importance, hyssop essential oil has been the object of many phytochemical investigations, but the intra-specific rank has been examined only sporadically. Generally, *H. officinalis* subsp. officinalis is mainly characterized by monoterpene ketones such as pinocamphone and isopinocamphone, and by smaller amounts of β-pinene (i.e. the biogenetic precursor of isopinocamphone), pinocarvone, limonene, 1,8- cineole, linalool and camphor [10].

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Many studies reported great chemical variability, attributing it to such factors as origin, harvesting time, genetic diversity and biotic stresses [11−15].

Antimicrobial and antifungal activities of the essential oil of hyssop have been reported in a number of studies [16−20], as well as anti-inflammatory effects [21, 22], but only negligible antioxidant activity has been reported [23]. These four activities were mostly observed in oil samples rich in ketones.

Supercritical fluid extraction using CO_2 as a solvent has been widely employed for several years in industry for obtaining active compounds from natural sources [24, 25]. Subcritical water is also employed for extracting active compounds such as polyphenols [26]. Supercritical CO_2 not only offers a cheap and environmentally friendly method for extraction processes, but it is also a selective solvent for certain natural active compounds as well. It is known that diverse parameters such as temperature, pressure, or the addition of an organic co-solvent may affect the CO_2 solvent strength, increasing the overall extraction yield and/or the composition of certain substances within the extract [27].

Many studies have examined supercritical extraction processes in order to determine extraction kinetics which are needed for developing optimal extraction models [28, 29]. These models can easily be applied to several supercritical extraction processes with very high accuracy. Generally, extraction kinetic curves present three different stages [29]. The first part corresponds to the easy extraction of accessible solute from superficial structures; the second presents a decrease in the accessible solute, and the last is the extraction of the least accessible solute. Extraction kinetics curves can also be applied to calculate solubility of the extracts in CO_2 [29].

From general principles of extraction with gasses under pressure, it is known that the value of pressure, at which the extraction is carried out, significantly influences on the extraction yield and composition of the extracts, [25]. Increasing pressure significantly increases the dielectric constant and the density of the solvent, and therefore the ability of extragents solubility. For these reasons, the extraction with gasses in supercritical state, provides a wide range of possibilities during extraction of hyssop [30].

From a large number of different gasses under pressure, which are applied as solvents for extraction of natural substances (fragrant, spicy and farmaceuticaly active substances) carbon dioxide has a distinct advantage. The power of dissolving of supercritical carbon dioxide depends primarily on the pressure and temperature, i.e. bulk density, which, for carbon dioxide on critical point amounts to 470.0 kg/m^3 [25]. By using carbon dioxide, it is possible to obtain extracts with the desired composition of components comprised in the starting material. For obtaining extracts by using supercritical carbon dioxide, there may be different procedures [25]:

− isothermal procedure (extraction is carried out at a constant temperature, while seperation of the extract by lowering the pressure);

− isobaric procedure (extraction is carried out at a constant pressure, while seperation of the extract by increasing the temperature);

− isobaric – isothermal procedure (extraction and isolation of the product is carried out at a constant pressure and temperature, and the product is isolated by adsorption on a suitable sorbent, for example, decaffeination of coffee and tea).

Several models have been proposed in the literature to describe the supercritical fluid extraction of oils and volatile oils. They are useful tools for the design, improvement and scale-up of these processes from laboratory to pilot and industrial scales. For the design and optimization of supercritical fluid extraction (SFE) processes, it is necessary to know the characteristics of the raw material (the initial concentration of the solute in the plant matrix, the composition of the solute mixture, the humidity and the pre-treatment of the raw material, such as drying and grinding), the process parameters employed in the extraction (pressure, temperature, solvent flow rate) and the fluid phase equilibrium data [31].

The available mathematical models to describe the extraction of solutes from solid matrices using supercritical fluids are classified in four main groups: empirical models, models based on heat transfer analogies, shrinking core model and models based on differential mass balances [32]. In this paper, we used the empirical models of Naiks.

2. MATERIAL AND METHODS

For this experiment hyssop is used (*Hyssopus officinalis* L.), grown in experimental plots of the Institute for hops, sorghum and medical plants in Bački Petrovac. For extraction, labaratory device High Pressure Extraction Plant was used (HPEP, Nova Swiss, Switzerland). The extraction process is performed by the plant material being milled and ground to a certain degree of fragmentation [25]. Preparation of the drug for extraction is carried out by fragmentation by using a commercial grinding mill (Multi Moulinex, 260W), taking care that the drug is not overheated during fragmentation.

For determining the granulometric composition of drug, a set of sieves by manufacturer Erweka Apparatebau GmbH (Germany) is used, [25]. As extragent, the commercial carbon dioxide of 99% purity is used, produced by Tehnogas (Novi Sad).

3. EXPERIMENTAL PART

Investigation of the effect of pressure of the solvent on the yield of total extract of hyssop, was performed at a pressure of 80, 100 and 150 bar, temperature T = 313 K, flow 0.00323 kg/min, by using drug of average diameter d = 0.49 mm, [22, 23, 25]. Drug (60 g) is carried into the extractor, and gaseous carbon dioxide was passed through it, at given parameters of extraction (pressure, temperature, flow rate of solvent). After the specified time of extraction duration, mass of the obtained extracts was measured and yield of extraction was calculated (g/100g of drug). Each point of the kinetic curve is obtained with a separate sample.

4. RESULTS AND DISCUSSION

The obtained results of supercritical extraction of hyssop at investigated pressures (80, 100 and 150 bar) are shown in figure 1.

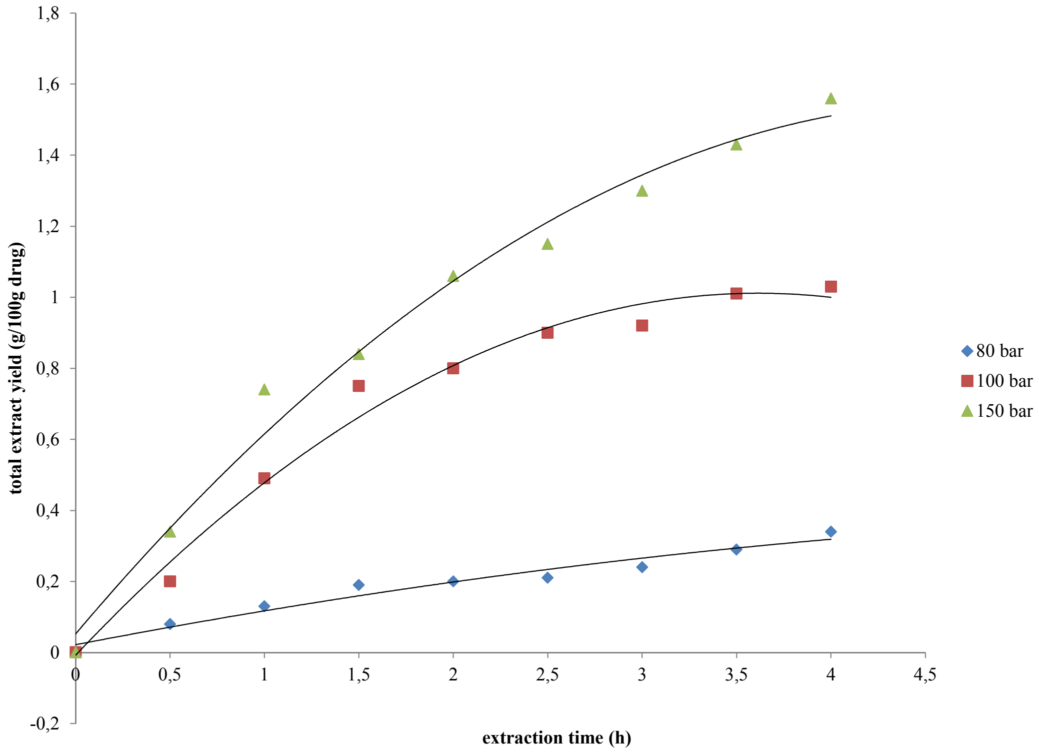

Figure 1. Graph of dependance of the yield of hyssop extract (g/100 g of drug) on time (extraction conditions: T = 313 K; w = $3.23 \cdot 10^{-3}$ kg/min; d = 0.49 mm).

The figure shows that the pressure of extraction significantly affects the yield of extract and at a pressure of p = 150 bar, it gives the highest yield, which is consistent with the theoretical fact that the power of dissolving the supercritical carbon dioxide primarily depends on the pressure. Durig the extraction under pressure of 150 bar and the extraction time of 4 hours under the above defined values of temperature, flow rate and degree of fragmentation, the obtained yield of extract was 1.56 g/100g of drugs, while at pressures 80 and 100 bar the yield was much lower (0.34 i.e. 1.03 g/100g of drug).

Within the given research, modeling of the extraction system of hyssop – supercritical CO_2 was performed, where empirical model of Naik and associates was applied. Naik and associates extracted several plant species (clove, ginger, fennel, dill) with liquid carbon dioxide and modeled the obtained results, [25]. They presented the yield of extraction, y, as a function of time, t, by an equation that is similar to Langmuir's adsorption ishoterm:

$$y = \frac{y_{\infty} \cdot t}{b + t} \quad (1)$$

where:

y – yield of extraction (extract) in time t,
y_∞ - maximal yield of extraction,
b – constant.

By transformation of the equation (1) it is obtained:

$$\frac{1}{y} = \frac{b}{y_\infty} \cdot \frac{1}{t} + \frac{1}{y_\infty} \quad (2)$$

By introducing the substitution t' = $1/t$, equation (2) is converted to:

$$\frac{1}{y} = \frac{b}{y_\infty} t' + \frac{1}{y_\infty} \quad (3)$$

Further, equation (3) is taking shape of line

$$\frac{1}{y} = Bt' + \frac{1}{y_\infty} \quad (4)$$

where:

$$B = \frac{b}{y_\infty}.$$

Model of Naik and associates is applied for following the yield of total extract of hyssop during 4 h extraction with supercritical carbon dioxide at various pressures. Results are shown in figure 2.

The obtained values of constant B (line slope) and $1/y_\infty$ (intercept on the ordinate) in equation (4), at different pressures are given in table 1.

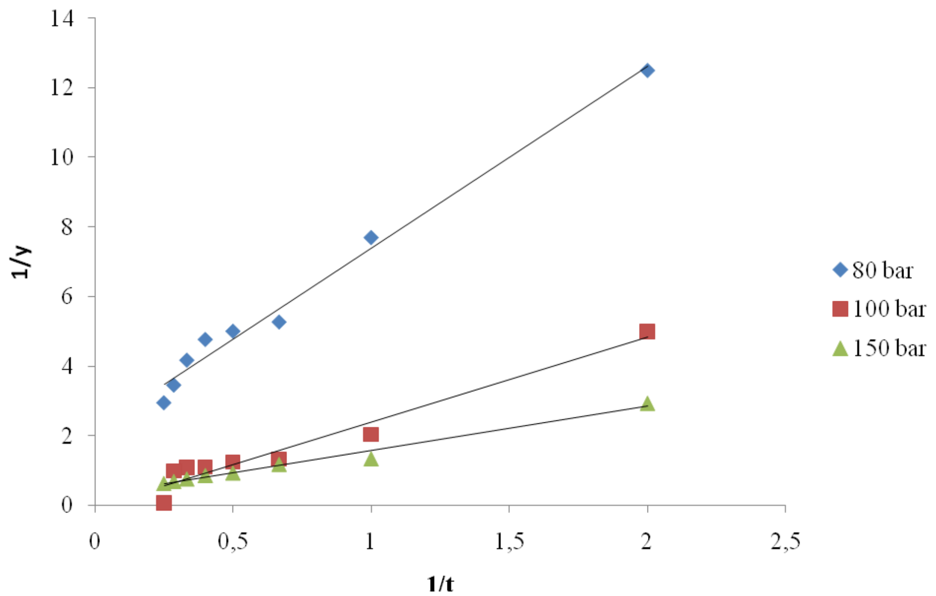

Figure 2. Dependance of the reciprocal value of yield on reciprocal value of extraction time for investigated pressures.

Table 1. The equations for calculating the yield of total extract of sage during extraction using model of Naik and associates

Pressure (bar)	Equation	|r|
80	1/y =5.220 t' + 2.174	0,992
100	*1/y* =2.445 *t'* – 0.051	0,976
150	1/y =1.271t' + 0.311	0,991

4. CONCLUSION

Based on investigation of kinetic extraction by measuring the yield of total extract depending on the pressure during the time of extraction four hours at given values of temperature, flow rate and degree of fragmentation of plant material, it is concluded that the highest yield of drug of 1.56g/100g is obtained at the highest pressure of extraction of 150 bar. The lowest yield of extraction (0.34g/100g of drug) was obtained at the lowest investigated pressure of 80 bar. Obtained results were in agreement with the theoretical fact that with increase of pressure, the power of dissolving the compounds in supercritical carbon dioxide rises.

Based on the high value of the correlation coefficient $|r|$ (0,976 – 0,992), it is concluded that during all applied pressures of extragent, a very strong correlation between the reciprocal value of total yield (1/y) and reciprocal value of extraction time (1/t) was obtained.

It was concluded that the model of Naik and associates, although applied to the extraction of natural material using liquid carbon dioxide, can be successfully applied at extraction of hyssop with supercritical carbon dioxide.

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ПРИМЈЕНА НАИКОВОГ МАТЕМАТИЧКОГ МОДЕЛА ЗА ОДРЕЂИВАЊЕ УКУПНОГ ЕКСТРАХОВАНОГ ПРИНОСА СИПАНА (HYSSOP) ДОБИЈЕНОГ СУПЕРКРИТИЧНОМ ЕКСТРАКЦИЈОМ СА УГЉИЧНИМ ДИОКСИДОМ

Сажетак*:* У овом раду врши се суперкритична екстракција сипана (*Hyssopus officinalis* L.) коришћењем угљичног диоксида као екстрактанта. Испитује се дјеловање притиска (80, 100 и 150 бара) на принос укупног екстракта при температури 313 К, протоку 0.00323 кг/мин и просјечном пречнику честица 0.49 мм. За моделирање екстракционог система сипан – суперкритични CO_2 користи се Наиков модел којим се одређује принос укупног екстракта. На основу вриједности коефицијента корелације |р| (0.976 – 0.992), закључено је да је добијена веома јака корелација између реципрочних вриједности укупног приноса екстракта и времена екстракције (1/у и 1/т), тако да се може закључити да се Наиков модел може успјешно примијенити за одређивање приноса укупног екстракта.

Кључне ријечи*:* сипан (hyssop), суперкритична екстракција, моделирање, угљен-диоксид.