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# Extraction of Glycyrrhizic Acid from Licorice Root using $CO_2$

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**ABSTRACT**: This study used a supercritical (SK) of $CO_2$-extraction to extract glycyrrhizic acid (GA) from the licorice root. To do this, define the conditions (preliminary experiments) of the extraction process, namely, the temperature, pressure and fluid flow SK ($CO_2$). Given that this process is multifactorial, the method RSM-response surface methodology and CCRD-central composite rotatable design used to determine the optimum operating conditions of the process. The effectiveness of the established SC-$CO_2$ extraction conditions, expressed GA content in the extracts as compared with a yield of GA produced by the conventional extraction method, when applied SC-$CO_2$ modified polar co-solvent (ethanol).

In describing the GA yield predictions using appropriately combined with RSM CCRD, we found that the yield of GA mainly depends on the pressure and quantity of SC-$CO_2$ used for extraction. It turned out that there is a significant relationship for the linear and quadratic terms of the relationship between the output of the GA and these parameters. Noticeable interaction between the three process parameters (pressure, SC-$CO_2$ temperature and flow rate) was observed.

Licorice root is subjected to moisture-heat pre-treatment. Cooked thereafter pitch used as a raw material for the extraction of GA by SC-$CO_2$ extraction. Initial studies for a wide spectrum of SC-$CO_2$ density value (780-890 kg/$m^3$) indicates that it is possible to set optimum operating conditions for the GA separation.

According to RSM-analysis of the optimal process conditions: 14.6 KPa, 33.5 $^o$ and 21.88 g $CO_2$/g.d.m. $CO_2$ consumption for the extraction of GA from licorice using SC-$CO_2$. SC-$CO_2$ density calculated for the optimum pressure and temperature equal to 885 kg/$m^3$, which was found as a result of a preliminary analysis of the correlation between the output of the GA and $CO_2$ density. The maximum yield of GA is equal to 0.158 g of 1 g of dried material (about 15% of extract) with SC-$CO_2$ density equal 863 kg/$m^3$.

Preliminary tests performed at condition resulting in SK-$CO_2$ density ranging from 780 to 890 kg/$m^3$ indicated that at some pressure, temperature as well as consumption of supercritical fluids the optimal working conditions for glycirhizin acid isolation could be determined. For thus purpose the following range of working conditions of SK-$CO_2$ were tested by using Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM): pressure from 16 to 34 KPa, temperature from 20$^o$ to 40$^o$ and consumption of SK-$CO_2$ from 10 to 26 *g*$CO_2$/*g*d.m. The results of this investigation indicated that maximum yield G.A. 158 mg from 1 g materials on dry basis (about 15% of total extract) at 14.6 KPa, 33.5 $^o$ and 21.88 *g*$CO_2$/*g*d.m. could be obtained.

**KEYWORDS**: extraction, supercritical $CO_2$, glycyrrhizic acid, licorice root, temperature, pressure.

## I. INTRODUCTION

In literature there are many ways to extract [1] of glycyrrhizic acid (GA) of liquorice roots, but none of them SC-$CO_2$ as the extractant was used. Advantages of SC-$CO_2$ extraction, as compared with conventional techniques already known [2]. SC-extract does not contain a solvent extraction and process can be accomplished very quickly and, finally, there is no need for further purification SC-extract from the extractant.

In this study, the supercritical $CO_2$ extraction used for extracting GA from liquorice root (licorice root, licorice). The purpose of the study was to assay the total yield of extract and GA content, which can be isolated under different conditions SC-$CO_2$ extraction. The first part of the research is dedicated to a detection influence of various SC-$CO_2$ density in the total yield of extract. Based on these preliminary data, as well as tests at different pressures, temperatures and fluid flow SC, tried to establish optimum operating conditions for the isolation of the GA by SC-$CO_2$ extraction. It was recently reported in the literature [3] on the application of RSM - response surface methodology and CCRD (central composite rotatable design) for these purposes. Efficacy installed SC-$CO_2$ extraction conditions, expressed as

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the content of diosgenin (DW) in the extracts, as compared with the output of DG obtained by traditional extraction (ethanol + water).
Moreover, studies on the effect of pressure, temperature, and SC-$CO_2$ flow to the output of the GA, as well as the interaction of these parameters during the process is not carried out until now. It appears that the selectivity of a $CO_2$ extraction can be achieved by using different-pressure, temperature and density of SC-$CO_2$ so as to return solubility SC-$CO_2$, which is directly dependent on the density of [3].

## II. EXPERIMENTAL PART

The traditional source of GA, which refers to the steroidal saponins and its molecule is very similar to the structure of the adrenal hormones (cortisone, etc.), and therefore is used in hormone replacement therapy, are the roots of liquorice (G lycyrrhiza glabral). Extract GC brownish-yellow, completely dissolve in water contains 5-18% of dissolved substances.
Roots were pretreatment water – head (105 ° C, 10 min.), which the aim of softening materials, and then prepare the pitch size of 0.3-0.5 mm and 0.4 mm thick.
Extraction was carried out in weighing bottles firm autoclave ASTELL (CK), the extracts were collected in receptacles to determine their total content. Samples were stored in the refrigerator (-5 ° C) for GC analysis by liquid chromatography of high resolution (HPTLC, ELSA company Ing - Techn 2000, UK).
Effect of SC-CO2 extraction was investigated at 40 ° C and 15 MPa. Samples were collected and after extracts of different extraction time, which corresponded to a specific SC-flow fluid. extract yield was compared and used for further analysis of the impact of operating conditions.
To investigate the influence of pressure, temperature and duration of extraction (SC-$CO_2$ flow rate), as well as their interaction are used RSM CCRD. A similar procedure was used previously [1]. However, instead of comparing the output diosgenin SC-$CO_2$ extraction was determined using a "overlapping" parameters (output DG received SC-$CO_2$ was compared with the yield by the method [2]) as a dependent variable [1], the total extract yield per gram cooked isolate Content and DG 100 g or isolate total 100 g of dry plants used in this study. Investigated parameters were pressure (denoted as $x_1$, MPa, temperature ($x_2$, C) and the amount of consumed $CO_2$ ($x_3$, m$CO_2$ / mGK). Actual and coded variables using in the experimental scheme, determined on the basis of initial tests carried out for different SC-$CO_2$ density. The central experience of building are 3 variables and all 20 experiments, including 9 factorial, 5 vertices and 6 central points for the RSM and CCRD analysis and used as shown in Table. 1. Polinomal equation 2nd order was considered to GC yield prediction as a function of independent (i = 3) pressure ($x_1$), temperature ($x_2$) and the amount of $CO_2$ ($x_3$) as encoded value

$$\gamma = \sum \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i^2 + \sum \sum \beta_{ij} x_i x_j$$

true for j <1 and i = 3.
Matlab version 2014 for the application of RSM and analysis of experimental data with a 3-dimensional surface and dependence «contourplots» independent variables and their inter-actions was used [7].

## III. RESULTS AND ITS DISCUSSION

### A. DRIVING EXPERIENCES

To extract glycyrrhizic acid (GA) of the licorice root with SC-$CO_2$ as an extractant in the following diagram of the experiments were examined.
Based on preliminary studies coded next network parameters ($x_1$, $x_2$, $x_3$) and uncoded parameters ($X_1$ - Pressure, MPa $X_2$ - temperature, °S and $X_3$ consumption SC-$CO_2$ g$CO_2$ / g d.m) were selected, as shown in Table. 1.

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**Table. 1.** Uncoded and coded values independent variables using in the RSM scheme

| The coded values | $P_1$ (MPa) | t, °S | Consumption $CO_2$ ($r_{CO2}/r_{d.m.}$) |
|---|---|---|---|
| -1.598 | 8.2 | 20 | 10.68 |
| -1 | 10 | 24 | 14 |
| 0 | 12.5 | 30 | 18 |
| +1 | 15 | 36 | 22 |
| +1.598 | 16.8 | 40 | 26.6 |

a) density SK-$CO_2$ 780-899.0 kg/m$^3$; b) t = 30 °S – central point, ±10 °; c) $P_{kr}$ = 7.41 MPa

Rotatable scheme applied to the above independent variables (pressure, temperature, CO2 loss), which resulted in 20 runs. Effect of SC-$CO_2$ density and time.

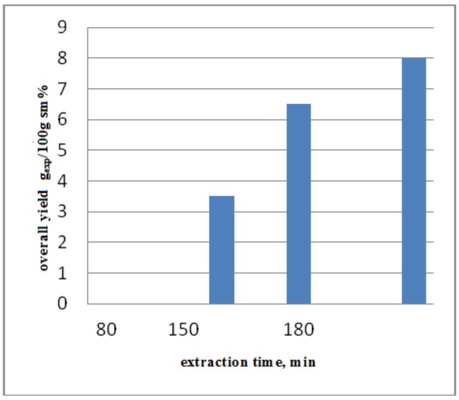


**Fig. 1.** The total yield of the extract as the feature extraction time, held at 20 MPa and 33 ° C

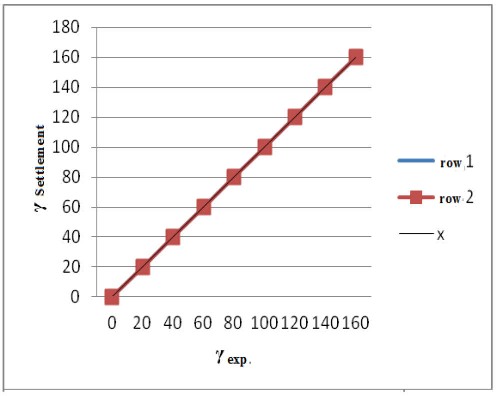


**Fig. 2.** Comparison of GA yield calculated using e quations 2 and 3, as founded experimental: $\gamma_{calc.}$ (2) $\gamma_{calc}$. (3) $\gamma_{exp}$.

Effect of SC-extraction time (15 MPa and 30 ° C) on GA yields shown in Fig. 1. It was found that the overall yield of extract can be increased by almost 2-times, while if SC-CO2 extraction increased from 80 min ($CO_2$ = 12 g$CO_2$ / g d.m) and 150 min (22 g $CO_2$ / g d.m) . Interestingly, if the proceeds to further increase the extraction time and ends after 180 minutes, it reached only the additional (10%) increase in the yield of the extract. This has been primarily for 150 min. SCE duration (rate of CO2 22 gCO2 / g d.m,) in which analyzed the total extract yield for different density 780-890 kg / m3). The results of these experiments are shown in Fig. 2.
Used for SC-$CO_2$ density above 800 kg / m3 of extract yield was more than 6% and about 880 kg / m3 attained maximum yield (7%). Although the relationship between the yield of the total extract and the density of SC-CO2 indicates that a maximum value of the output would be at a density of about 880 kg / m3, more realistic to assume that the yield could be higher for a density higher than 800 kg / m3 and that with increasing density above 880 kg / m3 total yield primary extract should be between 6 and 7% ($g_{exp}$ / g d.m 100).
According to preliminary experiments, although there were a limited number of (6), it is obvious that the desired optimum operating conditions for extraction can be set for SC-CO2 density higher than 800-840 kg / m3 and less than 880 kg / m3. This conclusion stems from experiments conducted at the same length (150 m) and further detailed and more accurate pressure and temperature analysis (including CO2 density) as well as the effect of SC-CO2 flow rate (i.e., the extraction time) carried out by using RSM and CCRD.

**B. RSM AND CCRD ANALYSIS**

Rotatable scheme used for the above three variables (pressure, temperature, $CO_2$ consumption), which is given 20 times. The results of determination of total extract yield are shown in Table. 2.

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**Table. 2.** The overall yield and allocation of GA using SC-$CO_2$ extraction, conducted under various conditions

| Pressure, (coded value) $x_1$ | Temperature (coded value) $x_2$ | Amount consumption $CO_2$ (kg/m$^3$) | Density $CO_2$ (kg/m$^3$) | yield extracts ($r_{exp}/r_{d.m.}$) | yield GK *r/r.d.m* |
|---|---|---|---|---|---|
| +1 | +1 | +1 | 890 | 90.1 | 134 |
| +1 | +1 | -1 | 890 | 80.2 | 120 |
| +1 | -1 | +1 | 947 | 80.2 | 126 |
| +1 | -1 | -1 | 947 | 70.3 | 104 |
| -1 | +1 | +1 | 781 | 60.8 | 101 |
| -1 | +1 | -1 | 781 | 60.1 | 92 |
| -1 | -1 | +1 | 859 | 70.3 | 101 |
| | -1 | -1 | 859 | 32.0 | 51 |
| +1.678 | 0 | 0 | 943 | 24.2 | 36 |
| -1.678 | 0 | 0 | 769 | 22.8 | 34 |
| | +1.678 | 0 | 821 | 100.4 | 158 |
| | -1.678 | 0 | 912 | 94.2 | 142 |
| | 0 | +1.678 | 876 | 94.2 | 142 |
| | 0 | -1.678 | 876 | 62.8 | 94 |
| | 0 | 0 | 780 | 90.4 | 140 |
| | 0 | 0 | 780 | 90.4 | 140 |
| | 0 | 0 | 780 | 90.5 | 141 |
| | 0 | 0 | 780 | 90.4 | 140 |
| | 0 | 0 | 780 | 90.2 | 139 |
| | 0 | 0 | 780 | 90.2 | 139 |

The influence of pressure, temperature and amount of $CO_2$ yield of GA and a quadratic function of the process parameters defined by equation (1) was tested. Using analysis of variance (ANOVA), calculated values of various coefficients that determine the yield of GA.

Linear and quadratic effect variables, and also their interaction and coe-patients, the value of the variables obtained by ANOVA calculated and displayed probable equation that is an empirical interaction between the output of the GA and independent variables (2).

$$\gamma = 0{,}70 + 0{,}033x_1 + 0{,}024x_2 + 0{,}073x_3 - 0{,}220{,}22x_1^2 + 0{,}001668x_2^2 - 0{,}064x_3^2 + 0{,}007802\,x_1x_2 + 0{,}000088x_1x_3 + 0{,}0008878\,x_2x_3 \quad (2)$$

where γ yield of GA (mgGA / g d.m) and $x_1$ - coded value of pressure, $x_2$ - coded temperature and $x_3$ coded value of the number of used SC-$CO_2$, as shown in the Table. 1.

Since the value of $R_2$ (0.9088) is greater than 0.8 which indicates that the model good confirms results.

Both linear and quadratic terms for the SC-$CO_2$ consumption had a greater loyalty ($p < 0.05$), as well as linear and quadratic terms of pressure. Interactions between the pressure, temperature and amount of $CO_2$ were not significant. Conducting RSM analysis showed the initial results and expectations, and that the pressure and SC-$CO_2$ consumption designated-as $x_1$, $x_3$ and $x_1^2$, $x_3^2$ terms were significant parameters of the model, which means that these independent variables (pressure and flow $CO_2$) could be used to determine the yield of GA with SC-$CO_2$ extraction.

Despite the fact that the extraction duration increases the yield of GA, it increases the cost of such an operation, and thus, an appropriate time may be set based on the results of this study.

The effect of temperature on the SC-$CO_2$ was observed after two possible effect on GA - extraction, although as mentioneding statistical analysis, the effect of temperature on the yield of GA was insignificant. Knowing that SCFE also designated as the process of "degradation", which means a combination of extraction and distillation, apparently high pressure SC-$CO_2$ was not favorable to the extraction (high density), while the high temperature increases the vapor pressure of the different compounds. However, according to the molecular weight of GA, as well as other compounds that could be extracted from liquorice, one would expect very little effect of temperature on extraction yield of GA. Thus, obviously GA has a low vapor pressure at the temperatures used in this study (30-50 °C) and RSM analysis showed this state, when the temperature (as a coded value of $x_2$) is almost insignificant parameter, which defines the

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output of GA SCF extraction (Equation 1 ). Furthermore, the temperature increase at constant pressure leads to a reduction in $CO_2$ density and thus reduces the effect of the solvent. Thus, the yield of HA increasing with increasing temperature analyzed, but noticeable effect is not detected.
Bearing in mind that the more important the independent variables with significant importance and influencing on the yield of HA are the pressure and flow SC-$CO_2$ and the following simplified equation could be used to calculate the isolation of licorice:

$$\gamma = 0{,}70 + 0{,}32x_1 + 0{,}073x_3 - 0{,}22x_1^2 - 0{,}064x_3^2 \qquad (3)$$

This equation can be used to output ($\gamma$) GA at pressures from 16.9 to 32.2 MPa (coded value $x_1$ -1.662 +1.668) and for flow rates of SC-$CO_2$ from 11.08 to 28.84 (also used as $x_2$ coded value from -1.688 to +1.668).
A comparison of the selected number of GA $\gamma$ calculated using equations (2) and (3) and the experimentally found values, $\gamma_{exp}$ is shown in Fig. 2. Shown only a small difference of correlation coefficient between calculated and experimentally determined values of $\gamma$ (r = 0.987 and using the equation (2), r = 0.989) is also used to show the results of the RSM-CCRD analysis.
It was shown that the amount of extract that can be obtained from licorice roots ranges from 8.17 to 16.80%. These results can be compared with the literature data, where extraction combined with microwave treatment and they accounted for 8.7%-16.8%. Some other literature data refer to SC-$CO_2$ extraction [4]. However, these authors use the term, defined as the ratio of SC-$CO_2$ output to what occurs when classical methods of extraction. They found that SC-CO2 extraction gives a yield of 20-85%, while in the classical method the yield ranges from 11-43%. You should also point out that SC-$CO_2$ extraction of licorice roots include vegetable processing [3], which was absent from the techniques used earlier in [4].
In accordance with the RSM analysis, optimum conditions for maximum yield GK (158 mghc/g d.m) are 14.6 KPa 38.8 $^{O}$S and 21.88 g $CO_2$/g d.m. use (or $CO_2$ for 150 min extraction). The density of $CO_2$ is calculated for the optimal values of pressure and temperature equal to 885 kg/m$^3$, which was also found in the result of the preliminary correlation analysis between the output of GK and the density of $CO_2$. As shown in the literature of optimum conditions for SC-$CO_2$ extraction are 14.6 KPa, $^{O}$S and 129.8 33.5 min (4.5) which is quite close to the optimal conditions we found.
Results SC-$CO_2$ optimization carried out in this study as well as those available in the literature for different plants proved that the temperature of extraction has little effect (or ignore) to the extraction (output) of the civil code. It is also important to note that this is the first study provides data on SC-$CO_2$ extraction of GA from licorice roots.

## IV. CONCLUSION

In the present study to describe and predict the yield of GA under different conditions SC-$CO_2$ extraction adequately used RSM in combination with CCRD. The yield of GA mainly depends on the pressure and quantity of SC-$CO_2$ used for extraction. It turned out that there is a significant relationship for the linear and quadratic terms of the relationship between the output of the GA and these parameters. There was no significant interaction between the three process parameters (pressure, temperature and consumption SC-$CO_2$). The optimal conditions: 14.6 kPa, 33.5° and 21.88 g $CO_2$ / g d.m. $CO_2$ consumption for the extraction of GA from licorice using SC-$CO_2$. The maximum yield of HA is equal to 158 mg of 1 g of dried material (about 15% of extract) may be carried out under certain optimum conditions, the extraction can be obtained at a maximum yield of GA-SC $CO_2$ density of about 863 kg / m$^3$.

1. Glycyrrhizic acid yield is mainly dependent on the pressure and quantity of supercritical $CO_2$ used for extraction. There is a significant relationship for the linear and quadratic terms of the relationship between the output glitsirrizines acid and these parameters. There was no significant interaction between the three process parameters (pressure, temperature and amount of supercritical $CO_2$).

2. The optimal conditions: 14.6 kPa, 33.5° and 21.88 g $CO_2$ / g d.m to obtain the maximum yield of glycyrrhizic acid, 0.158 g of 1 g of dry material.
Importance and influence on the yield of GA are the pressure and flow SC-$CO_2$ and the following simplified equation could be used to calculate the allocation of licorice.

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