

## Extraction of phytoecdysones with ethanol-modified supercritical CO<sub>2</sub>

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### Abstract

Biologically active polar substance 20-hydroxyecdysone was extracted from roots and leaves of *Leuzea carthamoides* with ethanol-modified dense carbon dioxide. The effect of operating conditions (pressure 20-27 MPa, temperature 40-60 °C, ethanol concentration in the solvent 4.3-7.1 wt.%, and solvent-to-feed ratio up to 100 g/g) on the extraction rate and the concentration in extract was examined. The maximum extraction yields were achieved with 7.1 wt. % ethanol in the solvent. Despite the high solvent-to-feed ratios the extraction was not complete; the maximum yield of 20-hydroxyecdysone from the leaves containing 1.50 mg/g dry mass was only 0.19 mg/g and the maximum yield from the roots containing 0.49 mg/g dry mass was 0.30 mg/g. The maximum concentration of 20-hydroxyecdysone in CO<sub>2</sub> extract from the roots, 2.9 wt.%, was higher than in the extracts obtained with 95% EtOH (1.74 wt.%).

Keywords: supercritical extraction, 20-hydroxyecdysone, *Leuzea carthamoides*, carbon dioxide, entrainer

### 1. Introduction

*Leuzea carthamoides* DC. (syn. *Rhaponticum carthamoides* [Willd.] Iljin (Asteraceae), a Siberian adaptogenic plant originating from the Altai and Saian Mountains (Lotočká *et al.*, 2004), has a woody rhizome with wiry roots and alternate, oval or elliptical leaves. The violet flowers appear in the second year. The plant was introduced to Central Europe as a medicinal crop (Zelený *et al.*, 1997) containing therapeutic compounds as ecdysteroids, flavonoids and N-feruloylserotonin isomers (Pavlík *et al.*, 2004). The major ecdysteroids in *L. carthamoides* are 20-hydroxyecdysone (20E), a polyhydroxylated, partly water soluble derivative of cholesterol, and further ajugasterone C (ajuC) and polypodine B (polyB). The content of 20E in dry seeds of *L. carthamoides* is as high as 2% (Stránský *et al.*, 1998). Fourteen minor ecdysteroids were isolated and identified from the roots of *L. carthamoides* by Vokáč *et al.* (2002).

Feeding Japanese quails with pulverized seeds of *L. carthamoides*, Koudela *et al.* (1995) confirmed previous findings of strong anabolic effects of the plant in growth of the vertebrate animals. Ibatov (1995) applied successfully decoction of the rhizome of the plant in therapy of alcoholic patients. According to clinical tests (Kormosh *et al.*, 2006), combination of ethanol/water extracts from adaptogenic plants (roots of *L. carthamoides*, *Rhodiola rosea*, *Eleutherococcus senticosus* and fruits of *Schizandra chinensis*) may boost the suppressed immunity in ovarian cancer patients who are subject to chemotherapy. Ethanol extract from *L. carthamoides* seeds has a strong repellent effect (Pavela, 2002).

20-Hydroxyecdysone is a hormone that plays a key role in insect development, cell proliferation, growth and apoptosis by controlling gene expression involved in molting and metamorphosis. Calas *et al.* (2006) examined the effects of 20E purified from *L. carthamoides* on the European grapevine moth and showed that it is sensed both in larvae and adults where it respectively inhibits feeding and oviposition. Pavela *et al.* (2005) tested on the cabbage aphid the effects of three ecdysteroid compounds isolated from *L. carthamoides*, 20E, ajuC and polyB, and found that 20E was less efficient than the other ecdysteroids, what concerns the mortality of larvae and adults.

In this work, the feasibility of supercritical fluid extraction of ecdysteroids from *L. carthamoides* was investigated. Due to the polar character of the extracted substances, ethanol was added to CO<sub>2</sub> solvent to increase its polarity.

## 2. Experimental

### 2.1. Materials

Air dried leaves and roots of *L. carthamoides* containing 5.8 and 6.1 wt. % of water, respectively, were milled and sieved; the fractions 0.2-0.4 mm size were used in all experiments. Carbon dioxide (> 99.9%) was purchased from Linde Technoplyn (Prague, CR), ethanol for spectroscopy was from Lachema Neratovice (CR). The chromatographic standards 20-hydroxyecdysone was from Sigma-Aldrich (Steinheim, Germany).

### 2.2. Supercritical extraction

The extractor of volume 12.6 cm<sup>3</sup> and inner diameter 8 mm contained 1.1-3.5 g of *L. carthamoides* leaves or roots placed between two layers of glass beads serving as flow distributors. Carbon dioxide from pressure bottle was pumped by a compressor, its pressure was adjusted by a pressure regulator and in a capillary it was mixed with ethanol pumped at constant flow rate by HPLC pump. The capillary was immersed together with the extractor in a thermostated water bath and connected to the extractor outlet. The solution of extracted compounds in the solvent flowed out of the extractor bottom to a heated micrometer valve where it expanded to ambient pressure. The compounds precipitating from gaseous mixture of CO<sub>2</sub> and ethanol were trapped in a glass U-tube and the amount of the gas flowing out of the trap was measured by gas meter. The amount of extract in the trap was determined gravimetrically. Three or

four samples of the extract were taken in each experimental run, changing the traps. The extraction conditions were the pressure in the range 20-27 MPa, the temperature 40-60 °C, the flow rates of CO<sub>2</sub> and ethanol 0.52 and 0.-0.037 g/min, respectively.

### 2.3. Extraction with ethanol

A sample (0.5 g of leaves or 1 g of roots) was mixed with 20 ml of 95% ethanol and boiled in water bath for 30 min. After 5 min. precipitation in was filtered through cotton wool, the residue was rinsed 2×3 ml ethanol and thr filtrates were joined. The residuum in the flask was mixed again with 20 ml of 95% ethanol and the procedure was repeated. Joint filtrates were filtered over diatomeous filter, the solution was concentrated, placed in an ampule, and the solvent was removed at 40 °C by a stream of nitrogen. The ampule was dried in a vacuum desiccator over silica gel for 48 h.

### 2.4. HPLC analysis

The HPLC separation and external standard method were applied for the quantitative determination of 20-hydroxyecdysone.

## 3. Results

### 3.1. Extraction yields of 20-hydroxyecdysterone

As proved by preliminary experiments, the extraction was equilibrium controlled; the yield was dependent only on the amount of solvent, not on the extraction time. The yield of 20E from the leaves was primarily influenced by ethanol concentration in the solvent (Figures 1, 2). The best results were obtained with 7.1 wt. % ethanol in the solvent at the maximum applied pressure. Even at the best conditions, the extraction of 20E was slow and the experimental runs were finished before the extraction was complete.

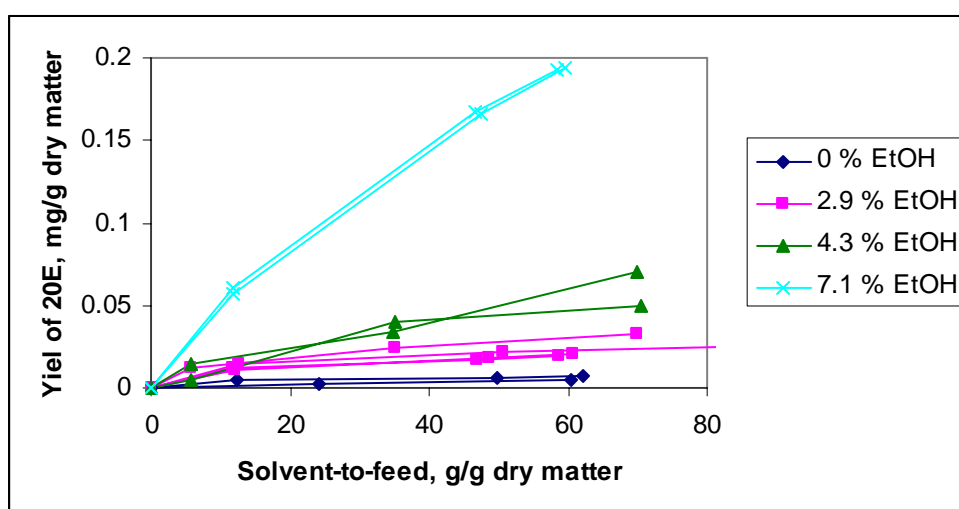


Fig. 1. Extraction of 20-hydroxyecdysone from the leaves. Extraction pressure and temperature: 25 MPa, 50-60 °C.

The rate of 20-hydroxyecdysone extraction from the roots was different; very slow extraction followed after a fast initial period, as shown in Figures 2 and 3. Thus, the yield in the end of the initial period was decisive for the final result. This yield increased with increasing ethanol concentration in CO<sub>2</sub>, pressure and temperature.

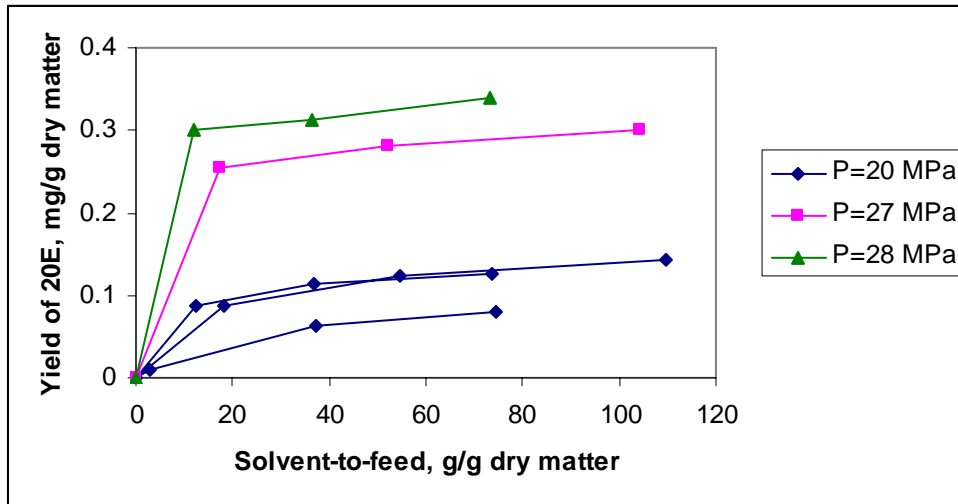


Fig. 2. Extraction of 20-hydroxyecdysone from the roots with 7.1 wt. % ethanol in the solvent at 60 °C.

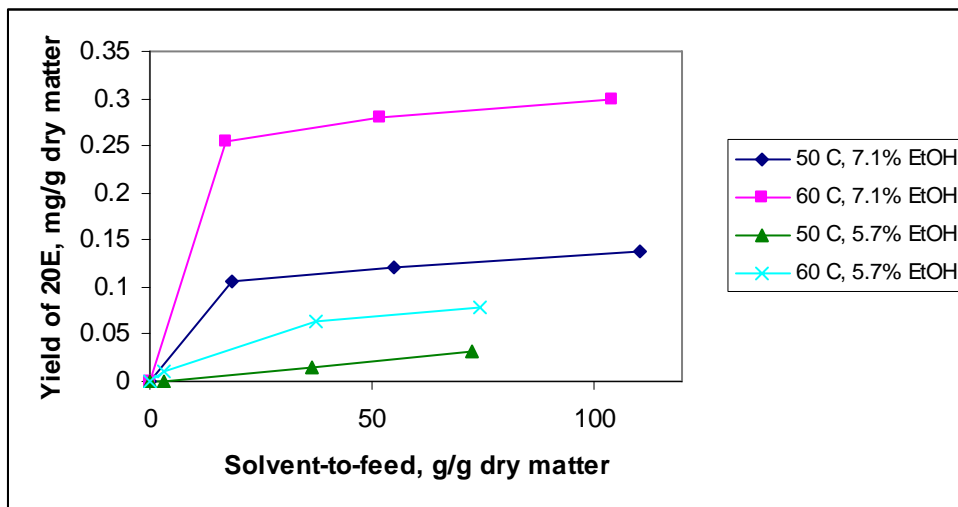


Fig. 3. Extraction of 20-hydroxyecdysone from the roots at the pressure 27 MPa.

In terms of the 20E yields, the present results of supercritical extraction are worse than those of the extraction with ethanol. As determined by the extraction with ethanol, the content of 20E in the leaves and roots was 1.5 and 0.49 mg/g dry matter, respectively, while the best results of supercritical extraction are 0.20 and 0.30 mg/g dry matter, respectively. However, the yield of 20-hydroxyecdysone in the supercritical extraction from both leaves and roots increased progressively with the

increase in ethanol concentration in carbon dioxide, and thus substantially higher yields could be expected at higher ethanol concentrations than 7.1 wt. %.

### 3.2. Concentration of 20-hydroxyecdysone in extracts

Generally, one of the expected advantages of supercritical extraction over the extraction with liquid solvents at ambient pressure is higher purity; it is higher concentration of required substances in the extract. In the present experiments the 20E concentration in the extracts obtained with ethanol from the leaves and roots of *L. carthamoides* were 0.71 and 1.74 wt. %, respectively.

In supercritical extraction from the leaves, the amount of total extract was dependent on the concentration of ethanol entrainer much less than the amount of extracted 20E. Besides, most of the total extract was collected at solvent-to-feed ratio less than 12 and then the amount of total extract was increasing only slightly, while the extraction of 20E continued in almost unchanged rate. Its concentration in the extract therefore increased with the yield of 20E, it is with increasing solvent-to-feed ratio and ethanol concentration in the solvent. The best 20E concentration in the extract at the solvent-to feed ratio equal to 56 was 0.52 wt. %. Again, an increase in the concentration is expected for higher ethanol concentrations in carbon dioxide in future experiments.

In supercritical extraction from the roots, the highest concentration of 20E in the extract, 2.95 wt. %, was also obtained for the maximum extraction yield of 20E.

## 4. Conclusions

The supercritical extraction of 20-hydroxyecdysone from *L. carthamoides* was controlled by phase equilibrium among solute, carbon dioxide, entrainer, and matrix, which was unfavourable for the extraction from leaves but acceptable for the first period of extraction from roots. The yield of 20E from the roots increases with increasing extraction pressure, temperature and content of ethanol in the solvent.

The yield of 20E from the roots at optimum extraction conditions (pressure 28 MPa, temperature 60 °C, ethanol concentration in the solvent 7.1 wt. %) was 0.34 mg/g dry matter, compared to the yield 0.49 mg/g dry matter obtained with ethanol. Its concentration in the supercritical extract, however, was 2.95 wt. %, compared to 1.74 wt. % in the extract with ethanol. Moreover, the extraction yields and 20E concentrations in the extract would further increase at higher ethanol concentrations in CO<sub>2</sub> than those applied in this work.

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