OPTIMIZATION OF OPERATIONAL CONDITIONS OF ETHANOL EXTRACTION OF ROSMARINIC ACID FROM LEMON BALM (Melissa officinalis L.)

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ABSTRACT
The process of ethanol extraction of a substance with antioxidant properties, rosmarinic acid (RA), from the plant Lemon balm (Melissa officinalis L.) is studied. The impact of some main process parameters (solvent concentration, process temperature and process duration) is experimentally determined, and the results helpful for process optimization are obtained. The general conditions for better practical extraction of RA from Lemon balm are defined.

Keywords: extraction, rosmarinic acid, Lemon balm

INTRODUCTION
Bioactive components are largely used in pharmacy, cosmetics, and perfumery or as nutritional additives. Substances of natural origin are preferred as substitutes of synthetic chemicals in order to reduce allergies and side effects. Consequently, studies on isolation of natural bioactive substances from plants contribute to development of processes with practical application.

Lemon balm (Melissa officinalis L.) has multiple positive actions on the human health due to its contents of natural bioactive substances [1-5]. This plant contains significant amounts of antioxidants (flavonoids, polyphenols) with main active component rosmarinic acid (RA) [1-4, 6].

Previous studies apply different kinds of treatment of Lemon balm with aim to isolate RA for studying its antioxidant activity, for comparison of its content in different plants, or for validation of different analytical methods [1, 3, 4, 6-11].
In this study, an experimental determination of optimized conditions for extraction of rosmarinic acid from Lemon balm is presented aimed at development of a practical process for production of RA using a harmless solvent ethanol.

**MATERIALS AND METHODS**

Rosmarinic acid has been chosen as a target component accounting for its recognized bioactive properties [7, 8, 12-15]. The choice of Lemon balm as raw material is due to its high content of rosmarinic acid and also to the fact that the plant is widely spread in nature and easy for cultivation [1, 3, 6-11]. Ethanol is chosen because of its frequent use as a harmless solvent for pharmacological and other applications. The goal of this study is to determine the process conditions, at which maximal amount of RA can be extracted from the raw material using ethanol as a solvent.

Solid - liquid extraction process has been used for isolation of RA from Lemon balm. Essentially, it consists of bringing together the raw material with the solvent, which dissolves the desired compound from the solid. All experiments have been made with dry plant at batch conditions. A weighted amount of dried and ground Lemon balm has been put in a glass flask. In order to operate at unsaturated concentrations, a measured amount of solvent has been added in large excess, and the flask has been continuously agitated for 24 hours in a thermostatic agitator. The samples withdrawn from the liquid phase have been filtered through a 20 µm micro-filter for elimination of solid particles prior to the injection into the analytical device (HPLC chromatograph).

**Analytical method**

RA concentration of the samples has been determined by means of high performance liquid chromatography (HPLC) with UV detector. Calibration solutions of RA in methanol have been prepared starting from pure RA (Fluka, purum > 95 %). A column Discovery® C18 (25cm X 4.6 mm, 5µ), Supelco has been used. The mobile phase has been methanol-water mixture 80:20 (v:v) with pH fixed at 2.5 using formic acid. The flow rate has been 0.4 ml/min and injection volume - 20 µl. All analyses have been carried out at ambient temperature. UV spectrum for the analysis of RA has been fixed at 280 nm [1, 11].

**RESULTS AND DISCUSSION**

The extraction yield \( w \) has been expressed as mass output, calculated by the following relation:

\[
w(\%) = \frac{m_{ex}}{m_{rm}} \times 100
\]

where \( m_{ex} \) is the extracted mass of RA in the sample calculated by the chromatographic analysis results, and \( m_{rm} \) is mass of the raw material.
**Influence of solvent concentration**

In order to determine the influence of solvent concentration, water solutions with various contents of ethanol have been used as solvents. The results are illustrated on Fig. 1.

![Figure 1. Influence of solvent concentration on extraction yield](image1)

Optimal yield has been obtained with ethanol diluted with water to 50 %. This result is clearly manifested by a distinct maximum at this value. The lower yield of more concentrated solutions can be explained by the increased solubility of other substances (chlorophyll and others) in concentrated alcohol, which engage the extraction capacity of the solvent.

**Influence of temperature**

The extraction process has been carried out at optimal solvent concentration and various temperatures taken in an interval from room temperature to temperatures below the solvents boiling point. The results are plotted on Fig. 2 and show a tendency for positive impact of increased temperature.

![Figure 2. Influence of temperature on extraction yield](image2)

However, it is seen that the yield does not vary significantly in the studied temperature interval, with particularly small change between 50 and 60°C.
Consequently, it is to recommend choosing operation at lower temperature (for example about 50°C), because such a regime will be with lower energy consumption and reduced risks for thermal destruction of the extracted substance.

**Kinetic study**
This study has been made in order to determine the development of the extraction process in the course of time. Samples at different moments of process duration have been taken and analyzed. Fig. 3 illustrates the results.

![Figure 3](image-url)

**Figure 3. Extraction yield in the course of time**

Two periods with different kinetic mechanisms might be clearly identified. Initially, the process velocity is high, which results in fast increase of RA concentration. After some time the mass transfer rate becomes slower, and the curve becomes nearly parallel to the abscissa, indicating negligible further concentration changes.

During the first period of fast mass transfer, the majority of the target substance is extracted. Consequently, the kinetic curve can serve to determine the process duration, which in this case is about 90 min.

**CONCLUSION**
An experimental study on ethanol extraction of rosmarinic acid from Lemon balm is carried out. The main operational conditions are varied in order to determine their impact on the process efficiency. As a result, the optimal solvent concentration, suitable process temperature, and duration of the process of extraction of rosmarinic acid from Lemon balm are determined.

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REFERENCES