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## Oleuropein extraction using microfluidic system

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#### ABSTRACT

In the present study, microfluidic devices have been used for extraction of oleuropein from ethyl acetate into aqueous phase. The effects of aqueous phase pH, temperature, flow rate ratio and residence time were investigated and optimized. Deionized water as extractant phase, ambient temperature, flow rate ratio of 1 and residence time of 0.1293 min were chosen as the best conditions. The content of oleuropein was determined by using high-performance liquid chromatography (HPLC). The extraction yield of 68.7% was obtained under the optimum conditions with relative standard deviation of 1.2%. The experimental results of microchannel extraction were compared with the batch process. The results revealed that the extraction yield using microchannel extractor device was more than that of conventional batch process. The results illustrate that the proposed technique has some advantages comparing with other methods including; simplicity of operation, cost effective and environmentally friendly.

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#### **1. Introduction**

Using liquid-liquid extraction (LLE) for concentrating and extracting analyte and also eliminating matrix interference is common in industrial and analytical processes. Since the conventional method of LLE process has some problems such as time consuming, requiring large amount of solvent, possibility of emulsion formation and losing analyte, finding a new method by using microchannels has become a major interesting field for industrial application [1]. It is obvious that, the less molecular distance leads to the more molecular diffusion [2]. Therefore, by decreasing the molecular distance the possibility of mass transfer will be increased. Increasing in surface to volume ratio is one of the most significant differences between micro and large scale devices. Therefore, miniaturization can be an effective way to enhance heat and mass transfer rate. Moreover, in this type of process, the volume of solvents and process time can be decreased as well as the cost of mass production [3,4]. Nowadays, the microchannel devices are widely used for various applications such as synthesis of organic molecules [5], synthesis of nanoparticles [6], kinetic studies [7,8], emulsification [9], solvent extraction [10–12], DNA extraction [13], solid-phase extraction [14,15], laser reaction

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http://dx.doi.org/10.1016/j.cep.2015.03.023 0255-2701/© 2015 Elsevier B.V. All rights reserved. control [16], immunoassay [17], flow-injection analysis [18,19] and biofuel processes [20–22].

Liquid–liquid extraction processes by using microfluidic devices have been reported in some literatures. For instance, the extraction of vanillin from water into toluene was reported by Assmann and Von Rohr [23]. In this study, an inert gas was used to enhance the extraction efficiency. The mass transfer rate in extracting succinic acid from *n*-butanol to aqueous drops containing NaOH by using microfluidic devices, was 10–1000 times higher comparing with the traditional liquid–liquid process [24]. Extraction of Fe-bathophenanthrolinedisulfonic acid complex from aqueous phase into chloroform is another example of quick extraction by using microchannel devices [10].

Pharmaceutical applications of natural materials from plants are backing to ancient times [25]. Oleuropein is one of the compounds that exist in olive leaves, fruit, bark and roots of *Olea europaea* (the olive tree) which causes bitter taste of olive oil and fruit [26,27]. It belongs to a group of compounds that is known as polyphenols. In the exposure of enzyme, oleuropein decomposes to enolic acid and hydroxytyrosol. Oleuropein has beneficial properties in medical products including; antibiotic [28], antimicrobial and antifungal [29,30], antimalarial [31], using in functional dairy products [32], food antioxidant [33,34] and prevention of Alzheimer [35]. These beneficial properties lead to seek efficient method for separation and purification of oleuropein. The most common problem in extraction is usage of toxic extractants such as mixture of methanol–water [36] or methanol-hexane [37]. Extraction by resin is another method. For example, using macroporous resins for simultaneous separation and purification of flavonoids and oleuropein from olive leaves was reported by Li et al. [38]. Usage of resin is time consuming and needs numerous steps. It seems necessary to find a simple, safe and cost effective method for extracting and purifying oleuropein.

The main aim of this study is to introduce and develop the microfluidic devices for extracting oleuropein from organic to aqueous phase. Liquid–liquid extraction using microfluidic system can be an effective method to perform a continuous process as well as lower solvent consumption. This study proposes a safe extraction method by using water as an extractant. The influences of different parameters on extraction yield were investigated and optimized.

#### 2. Experimental section

#### 2.1. Solvent extraction

For solvent extraction, 10g of air-dried and pulverized *Olea europaea* leaves were extracted by mechanical stirring for 24 h with 100 mL ethyl acetate. The supernatant phase was separated by a filter (Whatman filter paper). Consequently, this phase was used as a feed for microfluidic extraction.

#### 2.2. Microfluidic extraction

A schematic view of the microfluidic device with other equipment using in this study is shown in Fig. 1. The main part of this device is a T-shaped microchannel mixer. The geometric dimensions of T-shaped microchannel were 2.3 mm, 800  $\mu$ m and 8.5 mm in outer diameter, inner diameter and length, respectively. In order to increase the residence time, a coil with an outer diameter of 2.0 mm, an inner diameter of 600  $\mu$ m and length of 900 mm was used in the outlet stream. The syringe pumps were used to divert feed in each inlet stream (ethyl acetate extract and aqueous phase). The microchannel was placed in a water bath to keep its temperature constant at the selecting range, during the experimental process. Since the applied phases are immiscible with different density (897.00 kg/m<sup>3</sup> for ethyl acetate and 999.97 kg/m<sup>3</sup> for water), the aqueous phase was separated from organic phase by using a syringe. After separating two phases in the outlet stream, concentration of oleuropein was determined by using HPLC system. In order to find the optimum extraction conditions, the effect of various parameters such as pH, temperature, flow rate ratio and residence time were examined. The results were reported in terms of extraction yield percentages and compared with those the batch process.

The extraction yield was calculated according to Eq. (1) as follow:

$$Yield(\%) = \frac{m_a}{m_t} \times 100$$
(1)

where  $m_a$  is the oleuropein content (mg) in aqueous phase and  $m_t$  is the total content of oleuropein in feed. In all diagrams, error bars are evaluated in terms of relative standard deviation (RSD %).

#### 2.3. Chemicals and materials

Oleuropein (purity  $\geq$ 98% by HPLC) was purchased from Indofine Chemical Company (Hillsbrough, USA). Acetonitrile (HPLC grade), ethyl acetate, methanol, sodium hydroxide, dibasic sodium phosphate, potassium dihydrogen phosphate and orthophosphoric acid were purchased from Merck Chemical (Darmstadt, Germany). All solutions were prepared using deionized water from a Milli-Q system (Millipore, USA).

#### 2.4. Samples

Olea europaea (variety Sevillana) leaves were collected from Agricultural Research Garden, Khorramabad, Iran. Before the extraction, the leaves were washed and dried, then milled, homogenized and kept at  $4^{\circ}$ C until analysis. The same sample was used in the whole optimization study.

#### 2.5. Standard solutions preparation

A stock standard solution (4000 mg/L) was prepared by dissolving oleuropein in methanol. Working standard solutions at concentration of 500–2000 mg/L were prepared by diluting the suitable volume of the stock standard with deionized water.

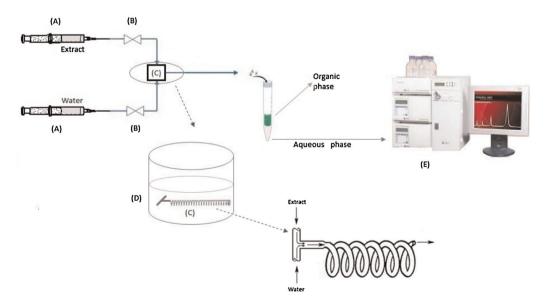


Fig. 1. A schematic diagram of the experimental setup: (A) syringe pump, (B) valve, (C) microchannel, (D) water bath, (E) HPLC system.

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