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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.

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 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

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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, 10 g 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 μm 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 μm 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^3 for ethyl acetate and 999.97 kg/m^3 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:

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

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 ≥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 °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.

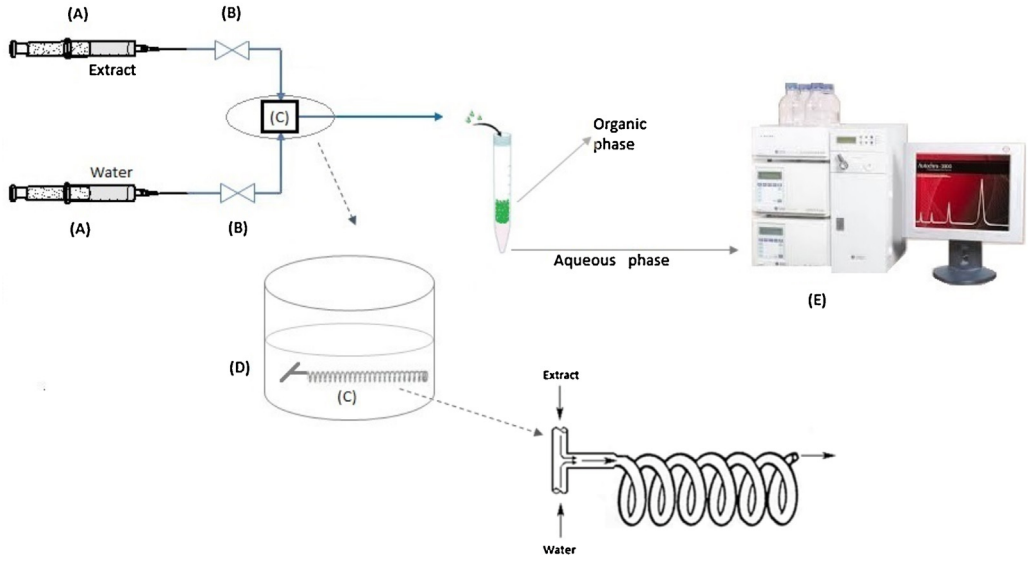

Fig. 1. A schematic diagram of the experimental setup: (A) syringe pump, (B) valve, (C) microchannel, (D) water bath, (E) HPLC system.

Oleuropein content (mg) in aqueous and organic phases was found using the prepared calibration curve.

2.6. Chromatographic conditions

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of a quaternary pump (LC-10ATvp), UV–vis detector (SPD-M10Avp), vacuum degasser and system controller (SCL-10Avp) was used. A manual injector with a 10 μL sample loop was applied for loading the sample. Class VP–LC workstation was employed to acquire and process the chromatographic data. A reversed-phase C_{18} analytical column (Shim-Pack VP-ODS, 250 mm × 4.6 mm i.d., Shimadzu, Japan) was used.

The mobile phase consisted of phosphate buffer (0.05 mol/L and pH 3 adjusted with orthophosphoric acid) and acetonitrile (70:30, v/v). Prior to preparation of the mobile phase, the buffer solution and acetonitrile were degassed separately using a Millipore vacuum pump. The UV detector was set at 254 nm. The chromatograms were run for 10 min at a flow rate of 1.0 mL/min at ambient temperature.

3. Results and discussion

In the first extraction step, by considering selective extraction capability of ethyl acetate and green chemistry (low toxicity, environment-friendly solvents), ethyl acetate was chosen as extractant. In despite of the fact that there is slight polarity difference between oleuropein and flavonoids, ethyl acetate is able to extract oleuropein more than flavonoids [38]. Also, according to Food and Drug Administration (FDA) solvent classification, ethyl acetate belongs to low toxic solvent, which can be used in food and pharmaceutical industries [39]. In the present work, the influential variables on the extraction efficiency of oleuropein such as pH of extracting solvent, temperature, flow rate ratio, and residence time were studied and optimized.

3.1. The pH effect

In order to evaluate the effect of pH on extraction efficiency, the phosphate buffer (dibasic sodium phosphate 0.05 M) with different pHs in the range of 2–10 including; 2, 4, 5, 6, 7, 8 and 10 were used as extractant phase. In this step, fixed values of temperature, flow rate ratio and residence time were used. As it can be seen in Fig. 2, increasing the pH value from 2 to 7 leads to increase in the yield of extraction, whereas the extraction yield has decreased by increasing of pH from 7 to 10. Therefore, the pH value of 7 was selected as the optimum value, which is compatible with another report in literature [40].

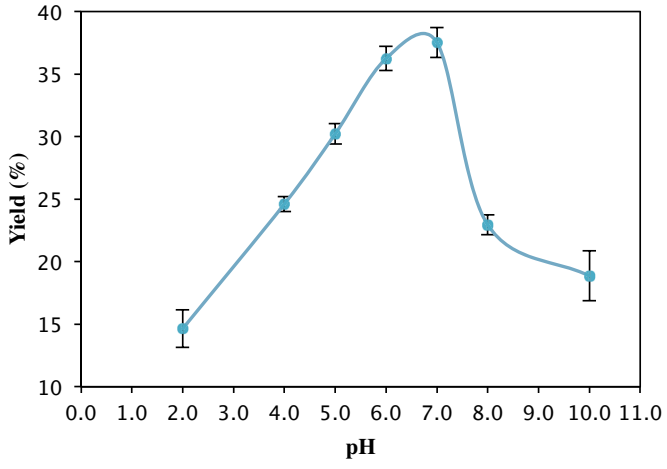

Fig. 2. pH effect on extraction efficiency. Extraction conditions: ambient temperature; flow rate ratio: 1; total flow rate: 2 mL/min; residence time: 0.0021 min.

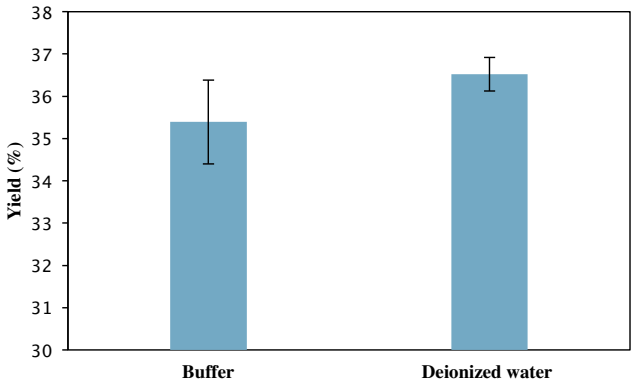

Fig. 3. Comparison of phosphate buffer pH 7 and water as extractant. Extraction conditions: ambient temperature; flow rate ratio: 1; total flow rate: 2 mL/min; residence time: 0.0021 min.

Since the maximum extraction was obtained in the neutral pH, then deionized water was used instead of phosphate buffer in the subsequent experiments. The results are illustrated in Fig. 3. Extraction efficiency using water as extracting solvent was higher than phosphate buffer (pH 7). This behavior can be attributed to ionic strength of phosphate buffer, which can decrease the mass transfer to extractant phase.

3.2. Effect of temperature

Temperature by changing and modifying the solvent and solute properties has a significant effect on extraction process. Temperature could provide the necessary energy to make the target components available for solvent extraction. Therefore, it is expected to increase the extraction efficiency by increase in temperature. In order to examine the temperature effect, the microchannel was placed in a water bath at three different temperatures including; 20, 40 and 60 °C. In this step, the experiments were carried out under the following conditions: a flow rate ratio of 1, a total flow rate of 2 mL/min and residence time of 0.0869 min. The obtained values of extraction yield were 61.2%, 62.06% and 62.13% at 20, 40 and 60 °C, respectively. Therefore, the results show that temperature has not any significant effect on extraction yield. This can be explained by the high contact surface between two phases in microchannels in which the mass transfer mainly performed by molecular diffusion. Therefore, the effect of temperature in this process becomes insignificant in comparison with large scale process. Therefore, ambient temperature was selected for further studies, which can be important from energy consumption point of view.

3.3. Effect of flow rate ratio of ethyl acetate/water

Flow rate ratio is one of the controlling parameters in the extraction rate [41]. In order to examine this parameter, different flow rates of input streams at the constant total flow rate were used. In this study, the flow rate ratio of ethyl acetate/water was varied from 0.5 to 3. In the large scale extraction, reducing the feed to extractant phase ratio leads to increase in the extraction yield. Therefore, it is expected to see an increment response that shows maximum extraction yield at minimal ratio of feed to extractant phase. In spite of that the reduction in the volumetric flow rate of extractant cause to decrease in extraction yield, by changing the

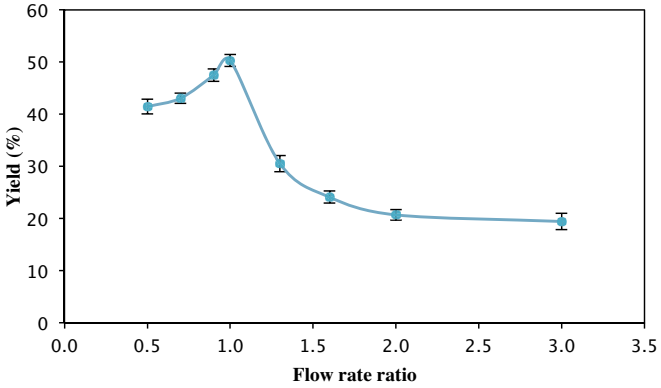

Fig. 4. Effect of flow rate ratio (ethyl acetate/water) on extraction efficiency. Extraction conditions: ambient temperature; total flow rate: 2 mL/min; residence time: 0.0445 min.

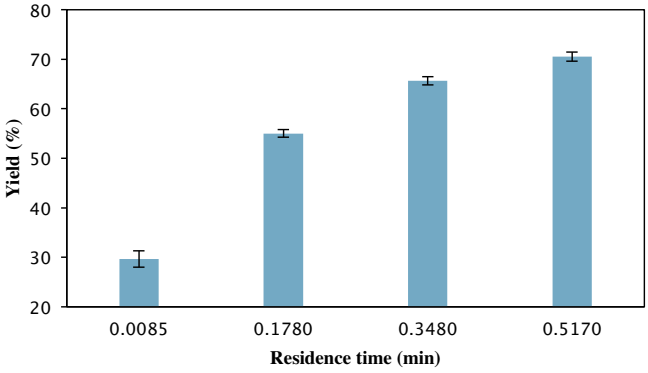

Fig. 5. Effect of residence time on extraction efficiency. Extraction conditions: ambient temperature; flow rate ratio: 1; total flow rate; 2 mL/min.

flow rate ratio from 0.5 to 1.0 the reduction in extraction yield was not observed (Fig. 4). This could be related to the effective collision in the junction of inlet streams. In the other hand, increase the possibility of mass transfer at the flow rate ratio of 1 due to presence of adequate volume of extractant is likely. According to these results, the flow rate ratio of 1 has been chosen for further experiments.

3.4. Effect of residence time

One of the important parameters that have a significant effect on the mass transfer is the contact time of two immiscible phases. In order to investigate the effect of residence time on extraction performance in the employed system, three different lengths of coil were used in outlet side and the results were compared with that of microchannel without coil. The obtained extraction yields are listed in Table 1. The experiments were accomplished in total flow rate of 2 mL/min. As it can be found in this table, the extraction yield is quite low (29.66%) when the plain channel was used. This result may not relevant for engineering applications, although it has low favor residence time. However, by increasing the coil length a more acceptable yield, up to 71.54%, obtained in just 0.1293 minutes. This is shown more clearly in Fig. 5, increasing the residence time culminates in increase the extraction yield. It is clear the longer contact time leads to the more mass transfer. Due to some limitations in syringe pumps power, no longer residence times were examined. On the other hand, a mass transfer analysis was undertaken to illustrate the effectiveness of increasing the coil length. In a simple form, the mass transfer rate equation can be expressed as follow:

$$N_{\text{oleuropein}} = K_{\text{overall}}(\overline{C}_{\text{org}} - \overline{C}_{\text{aq}}) \quad (2)$$

where $N_{\text{oleuropein}}$ is the molar rate of oleuropein transferred from organic phase to aqueous phase, K_{overall} is overall mass transfer coefficient, $\overline{C}_{\text{org}}$ and $\overline{C}_{\text{aq}}$ are the average oleuropein concentrations in organic and aqueous phases, respectively. The obtained values of overall mass transfer coefficient for three examined coil lengths

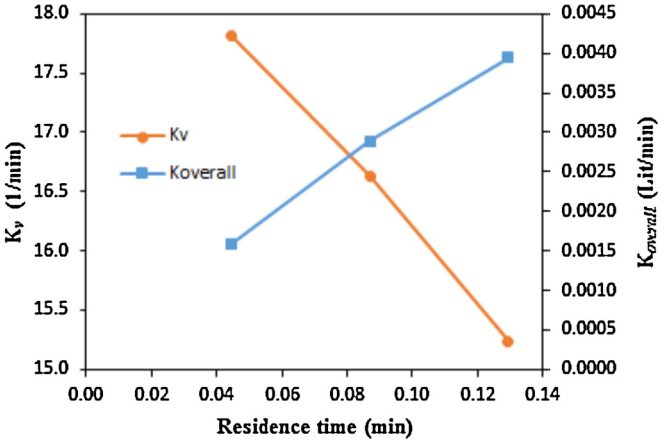

Fig. 6. The values of mass transfer coefficients at various residence times.

are illustrated in Fig. 6. The results show that the values of overall mass transfer coefficient increases with increase in the coil length that can be related to more time for mass transfer between two phases. However, as illustrated in Fig. 6, the values of the mass transfer coefficient per unit volume of the employed coil, K_v, have a reverse trend. The results show that the values of K_v decrease with increase in coil length. This means that the mass transfer between two phases is stronger at the beginning of the coil and decrease along the coil.

Finally, the longest time of 0.1293 min was selected as the best residence time according to the maximum power of employed syringe pumps.

3.5. Extracted oleuropein characterization

Typical chromatogram of oleuropein obtained by using microfludic device was shown in Fig. 7. The process conditions for this extraction were chosen according to the above mentioned optimum conditions. As it can be seen in the chromatograms, the retention time of oleuropein in extracting sample and standard

Table 1
Influence of coil length on residence time and extraction yield.

Coil length (mm)	Inside diameter (μm)	Outside diameter (mm)	Residence time (min)	Extraction yield (%)
–	–	–	0.0021	29.66[a]
300	600	2	0.0445	55.01
600	600	2	0.0869	66.64
900	600	2	0.1293	71.54

[a] Plain microchannel.

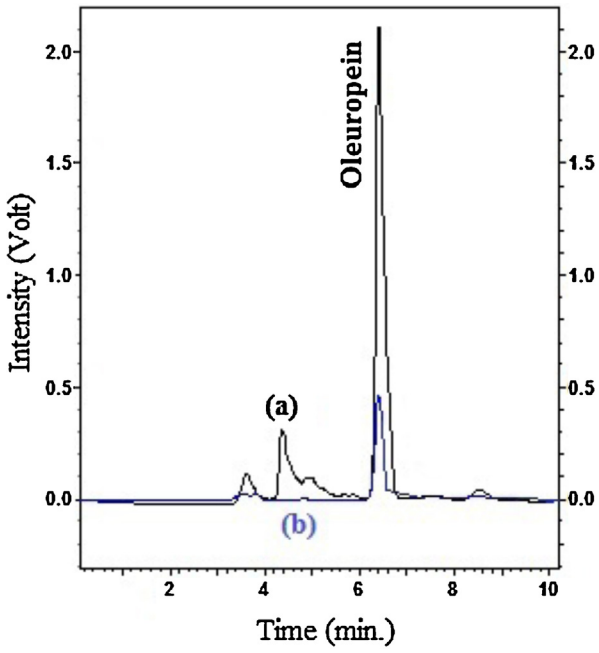

Fig. 7. Typical chromatograms of extracted oleuropein by microchannel device (a) and standard solution 500 mg/L (b). Extraction conditions: ambient temperature; flow rate ratio: 1; total flow rate: 2 mL/min; residence time: 0.1293 min.

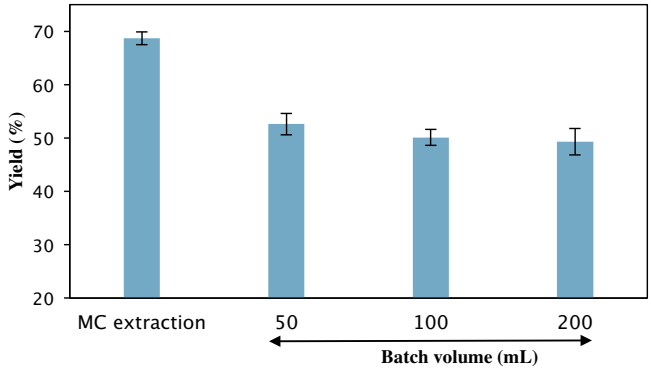

Fig. 8. Comparison of optimum microchannel (MC) and batch extraction.

solution is the same. This means that the employed system was successful in extracting oleuropein from feed.

3.6. Comparison between microfluidic and batch extraction system

In order to investigate batch process, three different volumes including 25, 50, 100 mL of ethyl acetate were mixed with the same volume of water in a beaker by using magnet stirrer for 45 min at ambient temperature. Considering the microchannel extraction results, the optimum conditions include flow rate ratio of 1 ambient temperature and water as extractant phase. The high efficiency of microfluidic extraction was proved by comparing the obtained results (Fig. 8). The extraction yield by microchannel device was approximately 18% more than the batch process extraction. By referring to the requiring time for the batch extraction (45 min) versus that of microfluidic extraction (0.1293 min), the importance of using this type of equipment is more obvious. Moreover, confident scale-up of this system by coupling many of them in a cascade parallel layout is another advantage of them, while there is lot of problem in scale-up of large scale batch process due inefficient mixing.

4. Conclusions

The reported extraction method represents the capability of mass transfer between immiscible liquids by means of microfluidic device. This continuous method of oleuropein extraction provides an environmentally friendly and high efficiency method in comparison with conventional techniques. In order to find the optimal conditions, the effects of different parameters including pH, temperature, flow rate ratio, and residence time on extraction yield were investigated. It was found that the temperature has not a great influence on extraction efficiency in the range of studied temperatures in this work. The flow rate ratio of 1 was selected as the best ratio of feed to extractant phase and deionized water was chosen as extractant phase. Moreover, the results revealed that the efficiency was improved in comparison with that of batch process. The results show that batch extraction in 45 min had lower performance than that of microfluidic extraction using 0.1293 min residence time. Finally, it is expected that the proposed continuous method to be used for mass production in a parallel cascade layout.

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