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# Development of a microfluidic-chip system for liquid–phase microextraction based on two immiscible organic solvents for the extraction and preconcentration of some hormonal drugs

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ABSTRACT

In the present study, for the first time, an on-chip liquid phase microextraction (LPME) coupled with high performance liquid chromatography was introduced for the analysis of levonorgestrel (Levo), dydrogesterone (Dydo) and medroxyprogesterone (Medo) as the model analytes in biological samples. The chip-based LPME set-up was composed of two polymethyl methacrylate (PMMA) plates with microfabricated channels and a microporous membrane sandwiched between them to separate the sample solution and acceptor phase. These channels were used as a flow path for the sample solution and a thin compartment for the acceptor phase, respectively. In this system, two immiscible organic solvents were used as supported liquid membrane (SLM) and acceptor phase, respectively. During extraction, the model analytes in the sample solution were transported through the SLM (*n*-dodecane) into the acceptor organic solvent (methanol). The new set-up provided effective and reproducible extractions using low volumes of the sample solution. The effective parameters on the extraction efficiency of the model analytes were optimized using one variable at a time method. Under the optimized conditions, the new set-up provided good linearity in the range of 5.0–500 μg $L^{-1}$ for the model analytes with the coefficients of determination ($r^2$) higher than 0.9909. The relative standard deviations (RSDs%) and limits of detection (LODs) values were less than 6.5% (n=5) and 5.0 μg $L^{-1}$, respectively. The preconcentration factors (PFs) were obtained using 1.0 mL of the sample solution and 20.0 μL of the acceptor solution higher than 19.9-fold. Finally, the proposed method was successfully applied for the extraction and determination of the model analytes in urine samples.



## 1. Introduction

Liquid-liquid extractions are widely employed for sample preparation prior to the instrumental analysis to reduce complexity of the matrix and increase the sensitivity of the analytical methods [1–5]. These methods are usually conducted using large amounts of various toxic organic solvents. Moreover, these techniques are time-consuming and require large sample volumes.

Liquid phase microextraction (LPME) is a miniaturized sample preparation technique that only uses several microliters of an organic solvent to extract the analytes [6–14]. However, several milliliters of sample solution is often required for LPME. This issue is one of the main challenges during the analysis of biological samples such as blood, plasma and saliva.

In recent years, lab-on-a-chip (LOC) systems have been introduced to overcome this problem [15,16]. These systems are more preferable for on-site and rapid analyses. Compared to the other LPME techniques, chip-based extraction systems have numerous advantages, such as less consumption of the sample solution and other chemical reagents as well as high extraction efficiency. The last one is attributed to the increase in the interface area to the volume ratio and the decrease in the diffusion distance [16–22].

Although, many articles have been published regarding different modes of LPME systems, less efforts have been made on the chip-based and microfluidic LPME systems [23]. Sikanen *et al.*, for the first time, introduced a new method named droplet-membrane-droplet liquid-phase microextraction under the stagnant condition [16]. In this work, target analytes were extracted from the sample droplet, through the SLM, and into an acceptor droplet. Wägli et al. designed an interesting set-up for the microfluidic-based multiphase LPME to detect cocaine by infrared (IR) spectroscopy from saliva samples [24]. In another work, Ramos-Payán

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et al. introduced a new microfluidic-chip based LPME system for the analysis of some basic drugs [25]. Subsequently, the acceptor phase was either analyzed off-line by capillary electrophoresis, or on-line by UV detection or electrospray ionization mass spectrometry. In all of these chip-based LPME techniques, an aqueous-organic (two-phase) or aqueous-organic-aqueous (three-phase) system has been used. Moreover, a small interface area has been applied between the sample solution and the acceptor phase, which can affect the extraction efficiency.

In the current research, a new microfluidic chip LPME system with a high interface area between the sample solution and the acceptor phase was designed. Additionally, for the first time, a three-phase system using two immiscible organic solvents was applied. The presented chip device, followed by high performance liquid chromatography-ultraviolet detection (HPLC-UV), was used for the extraction and preconcentration of levonorgestrel (Levo), dydrogesterone (Dydo) and medroxyprogesterone (Medo) as the model analytes. The effects of different variables on the extraction efficiency of the model analytes were studied and optimized. Finally, the proposed method was successfully applied for the extraction and determination of Levo, Dydo and Medo in human urine samples.

## 2. Experimental

### 2.1. Chemicals and reagents

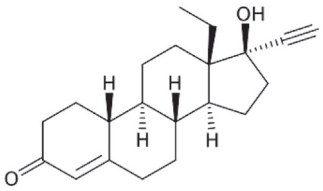
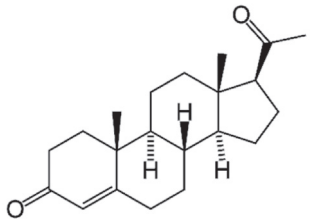
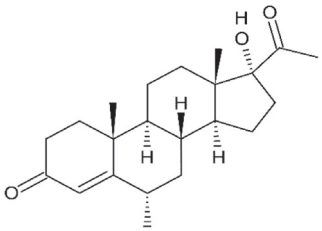
Levonorgestrel (Levo), dydrogesterone (Dydo) and medroxyprogesterone (Medo) were kindly donated by Aburaihan Pharmaceutical Company (Tehran, Iran). The chemical structures and properties of the drugs are shown in Table 1. Analytical grade *n*-dodecane and 1-octanol were supplied from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and methanol were purchased from Caldon (Ontario, Canada). The Accurel 2E HF (R/P) polypropylene membrane sheet was supplied by Membrana (Wuppertal, Germany) with a thickness of 150 μm, and a pore size of 0.2 μm. Ultrapure water was prepared by a Younglin 370 series aquaMAX purification instrument (Kyounggi-do, Korea).

Each analyte was dissolved in HPLC-grade methanol to obtain a stock solution with a concentration of 1.0 mg $mL^{-1}$, stored at 4 °C, and protected from light. Mixtures of diluted standard solutions were daily prepared by diluting the stock solutions.

### 2.2. Real samples

A human urine sample was collected from a 27-year-old healthy adult female volunteer in order to plot the calibration curves and to obtain figures of merit. Another urine sample was obtained from a 28-year-old healthy adult female volunteer. The urine samples were stored at −4 °C, thawed, and shaken before extraction. The sampling procedure was performed according to the

**Table 1**
Chemical structures and log *P* of the model analytes.

| Name | Chemical structure | Log *P* |
|---|---|---|
| levonorgestrel |  | 3.66 |
| Dydrogesterone |  | 3.79 |
| Medroxyprogesterone |  | 3.69 |

Ref. [34]

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