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Utilizing a Three-phase Hollow Fiber Liquid-phase Micro Extraction Method for Metoprolol Succinate Extraction from Biological Urine Prior to HPLC-UV Analysis

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This study presents a three-phase hollow fibers liquid-phase micro extraction (LPME) method for preconcentration of Metoprolol Succinate drugs from tap water and urine samples. The extracted analyte with LPME was detected with high performance liquid chromatography equipped with ultraviolet detector (HPLC-UV). The effect of several parameters on extraction efficiency was investigated and optimized, such as type of organic solvent, receiving phase, pH of receiving phase and extraction phase, extraction time and mixing speed. After extracting the Metoprolol Succinate drug under the optimum conditions, the pre-concentration factor was obtained 192 for tap water and 169 for urine samples with an appropriate detection limit of 3.90 μg l^{-1} for urine and 1.5 μg l^{-1} for tap water. The real sample study is provided satisfactory extraction recovery for urine (94%) and tap water (98%) samples.

Abbreviations

LPME, liquid-phase micro extraction; HPLC-UV, high performance liquid chromatography ultraviolet; LLE, liquid- liquid extraction; SPE, solid phase extraction; SPME, solid-phase micro extraction; HF-LPME, hollow fiber liquid-phase micro extraction; GC, gas chromatography; CE, capillary electrophoresis; SP, sample phase and RP, receiving phase

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Keywords: Hollow fiber liquid phase micro extraction, The human urine sample, Metoprolol Succinate, High performance liquid chromatography, Ultraviolet detector

INTRODUCTION

In recent years, researchers have been successful to introduce and develop faster and more accurate analytical methods particularly for analytical measurements of complicated biological and environmental samples [1,2]. First stage is sample preparation which is one of the most important steps in analytical procedures, as they directly affect the accuracy, the precision, the limit of detection and the extraction efficiency [3-5]. The liquid- liquid extraction (LLE) and solid phase extraction (SPE) techniques are the most common sample preparation techniques that are widely used in analytical approaches [6-8]. However, these techniques have some drawbacks such as low reproducibility, long extraction time and utilizing organic solvents leading to environmental pollution and health problems for human [9]. To solve these problems, researchers have introduced greener, simpler, faster and cheaper techniques [10-12].

Lately, by invention of the solid-phase micro extraction (SPME) by Pawliszyn *et al.* [10], new procedures have been introduced to micro extraction techniques in analytical chemistry that are applicable in nutritional, environmental and biological samples analysis [13,14]. The SPME technique provides potential benefits such as fast technique, consuming a low amount of polymeric material, fully

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automated, being an online method and solvent free with thermal desorption [10,13-15]. Although using the SPME fibers increasingly attract the scientists attention, it has some disadvantages including having a narrow range for desorption temperature (240-280 °C), being unstable in organic solutions, being unable to be coupled with HPLC, fiber destruction after utilizing for a couple of times, fragility of the fiber, syringe deflection and the high costs [16]. Therefore, to overcome these problems, liquid-phase micro extraction (LPME) have been introduced to analytical chemistry [17]. The LPME is the miniaturized size of LLE which has the effective analyte pre-concentration feature (source-phase) with only micro liters amount of the solvent (receiving- phase), besides, it does not have many of LLE's disadvantages [18,19]. The LPME is a simple, cheap and fast technique which typically needs no complex equipment, apparatuses and materials [20]. LPME is classified into three main classes: [17,18] single drop micro extraction (SDME), dispersive liquid-liquid micro extraction (DLLME), and hollow fiber liquid-phase micro extraction (HF-LPME).

SDME was developed by Liu and Dasgupta using an organic solvent micro drop (chloroform) for extraction of the analyte (dodecyl sulfate-methylen blue) from an aqueous sample. The SDME advantages include high concentration factor and good ability to load samples [21].

In 2006, for the first time, Asadi *et al.,* introduced the DLLME method based on two organic solvents one of which was extractant (immiscible) and the other was dispersive (missile) solvents. Hence, the high concentration factor and short extraction time are two advantages of this method [22]. However, these techniques suffer from some drawbacks such as being unable to extract from complex matrix and instability of the drop during stirring in SDME [23].

HF-LPME is another strategy in analytical extraction introduced by Bjegaard and Rasmussen in 1999 [24]. This technique can be coupled with high-performance liquid chromatography (HPLC) [25,26], liquid chromatography coupled with mass spectrometry (LC-MS) [27], gas chromatography (GC) [1,28] and capillary electrophoresis (CE) [29] for pre-concentration of analytes from environmental and biological samples. In addition to the high pre-concentration factor, sample cleaning-up, low solvent consumption and the ability to be automated [27] are the main advantages of HF-LPME.

The LPME can be classified into two groups: two-phase [30] and three-phase [31,32]. Two-phase micro extraction typically is performed by suspending a drop (some micro liters) of an organic solution on the tip of a Teflon spin bar or on the needle of a micro-syringe which is saturated with an aqueous sample [30]. The analytes are extracted into organic solution and then are directly injected into a GC or a HPLC process to be analyzed. In three-phase micro extraction (Fig. 1), the ionized analytes of an aqueous sample are extracted with a thin phase of an organic solvent within poly propylene hollow fiber pores or with an organic solution layer within a Teflon ring, then they are extracted back to receive aqueous solution [31]. As this method of extraction is performed in three steps, it has a higher selectivity compared to the two-step method [32-34]. Based on the literature, HF-LPME has a good ability to extract the drugs and metabolic compounds from the biological matrices and polluted environmental samples [34-36].

The aim of the present research is to develop HF-LPME technique for Metoprolol Succinate extraction and its quantitative detection in aqueous and urine samples prior to HPLC-UV analysis. Since Metoprolol Succinate is widely used in treatment of chronic chest angina, controlling its value in blood may help to prevent the angina and its symptoms. Thus, monitoring and quantitative analysis of the Metoprolol Succinate in blood or biological media are essential for health treatments. Additionally, the effective factors on the extraction efficiency were investigated and the optimum conditions were evaluated.

EXPERIMENTAL

Chemicals and Materials

Metoprolol Succinate drug with the highest purity based on national standards was purchased from Daroo-Pakhsh Company (Tehran, Iran). HPLC grade methanol and acetonitrile solutions, de-ionized water, glacial acetic acid, n-octhanol, n-dodecane, and n-endocanol were purchased from Merck Company (Darmstadt, Germany). Other chemicals such as sodium acetate, sodium dihydrogen phosphate, disodium hydrogen phosphate, ammonium chloride and ammonium hydroxide were purchased from

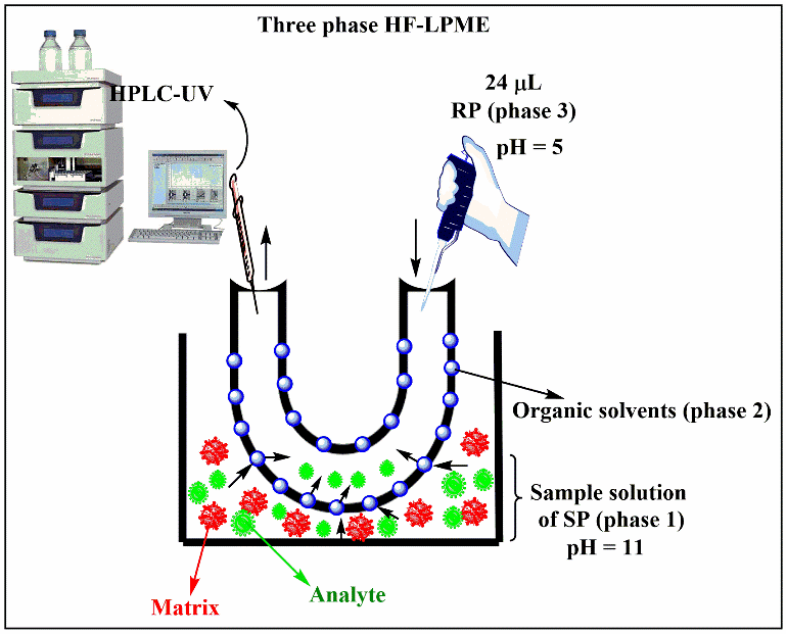

Fig. 1. Scheme of the three-phase hollow fiber liquid-phase micro extraction (HF-LPME) of Metoprolol Succinate from the aqueous media.

Sigma Aldrich Company (USA). Polypropylene hydrophilic capillary membrane (Type PP Q3/2, wall thickness 200 µm and inner diameter 600 µm and pore size of 0.2 µm) was obtained from Membrana GmbH (Wuppertal, Germany) and was used as hollow fiber polymer in the extraction process.

Preparing the Standard Solutions and Real Samples

The drug solutions were prepared in methanol with the concentration of 1000 mg l^{-1}. All standard solutions were used in fresh state and for the desired concentrations; working solutions were obtained by dilution of standard solutions with de-ionized water. All standard solutions were kept and stored in 4 °C temperature.

Instruments

The samples analysis was performed by HPLC instrument made by Agilent Company (model 1200). This set included one pump (model G1311A) with four solution entrances, ultraviolet visible (UV) detector (model G1314B) and one 20 µl loop. The HPLC consisted of a column by length of 150 mm and internal diameter of 4.6 mm and grains diameter of 5 microns (Model Agilent C18) that was used for separations. One dynamic phase containing of 60% volume of sodium dodecyl sulfate 0.1% in water and 40% volume of acetonitrile with flow rate of 0.9 ml min^{-1} in isocratic condition was used for sample washout; in addition, 223 nm wavelength was selected for detection.

The Extraction Procedure

As shown in Fig. 1, the polypropylene hollow fibers were cut in parts with length of 8.8 cm and immersed in organic solvents as extractant (n-octanol, n-endocanol and n-dodecane) to saturate the hollow fiber pores and channels. Then, hollow fibers were formed as a U shape. Thereafter, 24 µl distilled water (pH ~ 5) as a receiving phase (RP) was transferred inside the hollow fiber. Then, the end of hollow fiber was joined to the other needle tip. Additionally, 11 ml of water or urine samples (SP) was transferred to the glass vial (spiked and unspiked samples), pH was set at 11 and placed on magnetic stirrer. After the extraction, 20 µl of RP was injected into HPLC-UV by assistance of syringe (Fig. 1). All experiments were performed at room

temperature and the SP solution was stirred at 800 rpm for 50 min.

Calculations

The pre-concentration factor of extraction (EF) and the drug extraction percent were calculated as the following equations:

$$EF = \frac{C_{RP,final}}{C_{SP,initial}} \quad (1)$$

$$Extraction\ recovery\ (R\%) = EF \times \frac{V_{RP}}{V_{SP}} \times 100 \quad (2)$$

where $C_{RP,final}$ and $C_{SP,initial}$ are the final and initial concentrations of drug in RP and SP solutions, respectively. The $C_{RP,final}$ of the extracted drug was calculated from calibration curve. The V_{SP} and V_{RP} are the volumes of RP and SP solutions, respectively.

RESULTS AND DISCUSSION

The Main Rule of Extraction

In a three-phase micro extraction approach, the analyte was extracted into an organic phase located in fiber walls, and then transferred to RP solution located inside the fiber. For one analyte such as (A), the extraction process can be written as follows:

$$A_{SP} \leftrightarrow A_{MP} \leftrightarrow A_{RP} \quad (3)$$

The initial amount of analyte (n_i) is equal to sum of the special amounts of analyte thorough all phases in all extraction processes,

$$n_i = n_{SP} + n_{MP} + n_{RP} \quad (4)$$

where n_{SP} is the analyte amount in SP solution, n_{MP} is the analyte amount in membrane phase solution and n_{RP} is the analyte amount in RP solution. In equilibrium conditions the Eq. (3) will be as follows:

$$C_i V_{SP} = C_{eq.SP} V_{SP} + C_{eq.MP} V_{MP} + C_{eq.RP} V_{RP} \quad (5)$$

which C_i is the initial concentration of analyte, $C_{eq.SP}$, $C_{eq.MP}$ and $C_{eq.RP}$ are the analyte concentrations in SP, MP and RP solutions in equilibrium conditions, respectively, V_{SP}, V_{MP} and V_{RP} are the volumes of source, membrane and receiving phases, respectively.

Optimization of HF-LPME Method

Selection of organic solvent (extractant). Finding the compatible organic solvent as extractant to fill the HF-LPME polymer pores is critical since it affects the pre-concentration and extraction efficiency directly. The extractant should be inactivate, compatible with HF, insoluble in water and it should be able to be easily placed in hollow fiber pores. In this research, *n*-octanol, *n*-undecanol and *n*-dodecane were evaluated as organic solvents in HF-LPME technique as shown in Fig. 2. Based on the results, *n*-dodecane provided high extraction efficiency and it was selected as the organic phase for the next experiments.

pH of SP and RP. The effect of pH on extraction efficiency was studied for both SP and RP phases. As shown in Fig. 3, the pH of SP was changed in the range of 7-12 and extraction efficiency was monitored. As can be seen, the pH of SP at 11 provides a high efficiency. Additionally, at this pH, the Metoprolol Succinate is in its free form and can transfer the sample solution to the organic extractant. Moreover, in further experiments the pH of RP solution was studied in the range of 4-12, as shown in Fig. 4. It is obvious that at acidic pH the extraction efficiency is higher than the alkaline pH and the highest efficiency was obtained at pH 5. At the proposed pH, the Metoprolol Succinate is found in ionized form. Thus, based on pH gradient between SP and RP solutions, there is a driving force to transfer drug from SP to RP at pH 5.

The stirring speed. The principal of micro extraction process with hollow fiber is based on equilibrium between three phases [31,37]. Typically extraction speed is limited by mass transfer between SP phase, organic phase and RP phase (three phases) [37]. It is expected that by increasing the stirring rate of the sample solution, the mass transfer is improved from SP to RP solution. The effect of stirring rate on Metoprolol Succinate extraction efficiency was investigated in the range of 200-1000 rpm. As can be seen in Fig. 5, by increasing the stirring rate from 200 to 800 rpm the R% is increased from 30% to 60%. Thereafter, by the

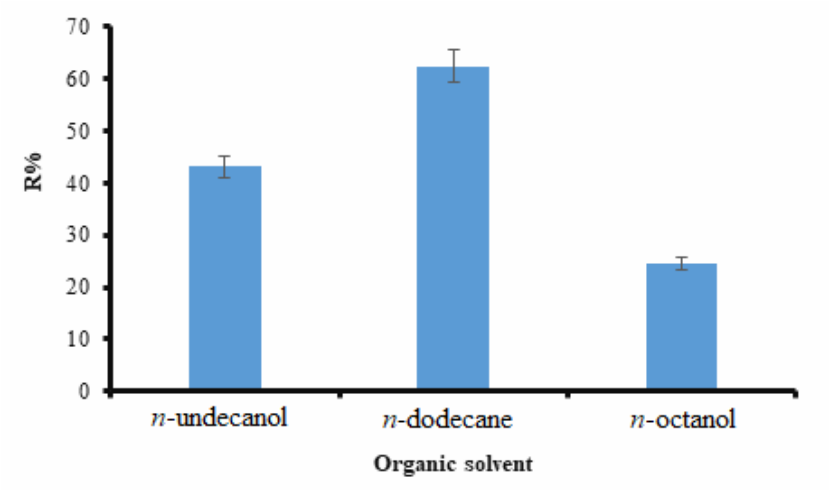

Fig. 2. Effect of organic solvent type on the extraction efficiency.

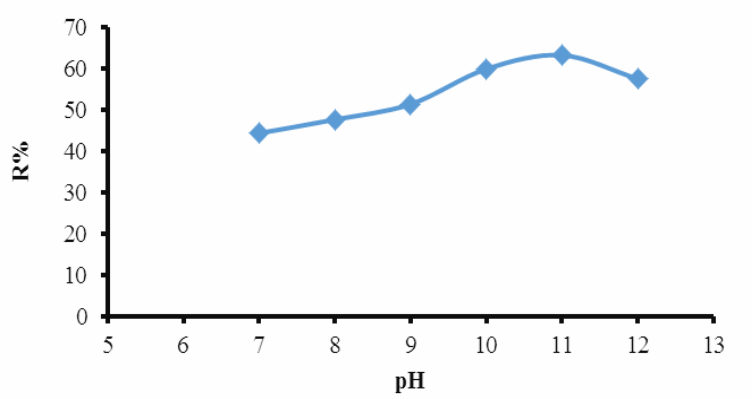

Fig. 3. Effect of SP solution pH on the extraction efficiency.

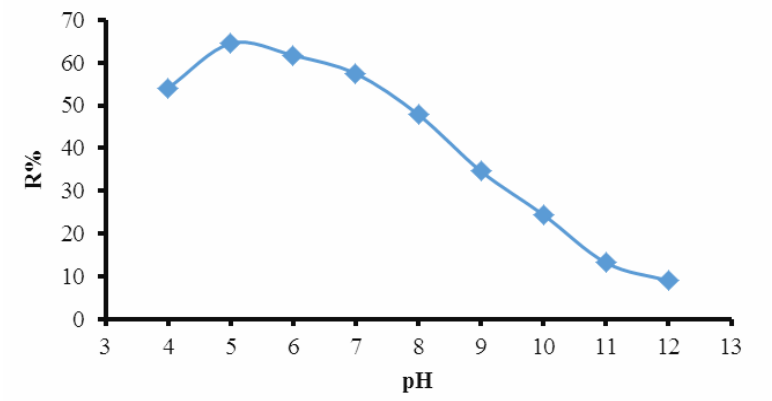

Fig. 4. Effect of RP solvent pH on the extraction efficiency.

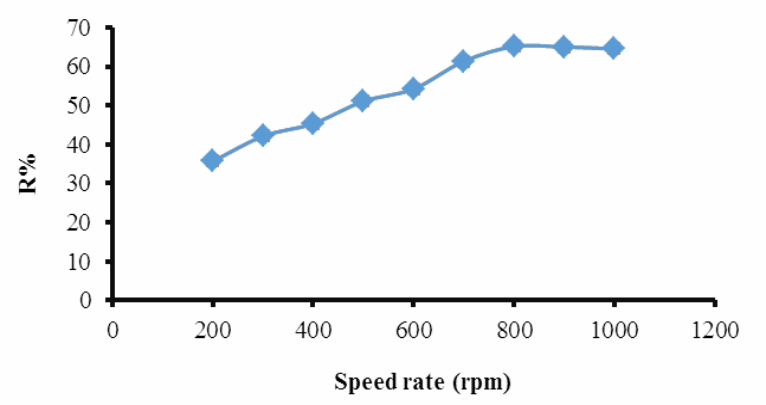

Fig. 5. The effect of stirring speed on the extraction efficiency.

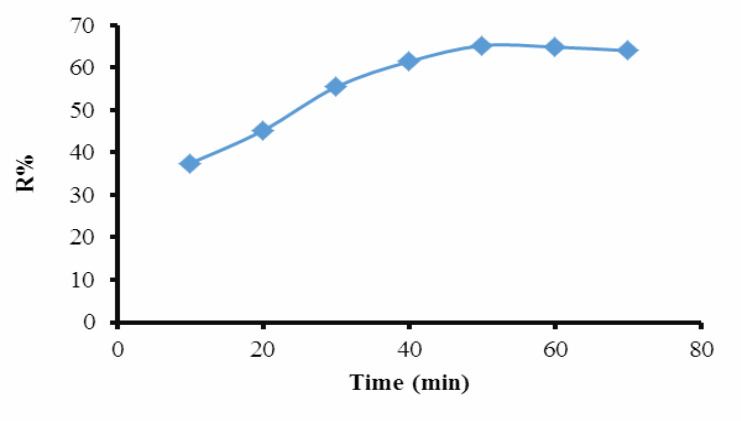

Fig. 6. The effect of time duration on the extraction efficiency.

Table 1. The Extraction Recovery in Real Sample for Metoprolol Succinate Drug

Samples	Parameters	Found	Recovery (%)
Tape water spiked ($5\ \mu g\ l^{-1}$)	Native concentration	ND[a]	-
	Determined concentration	4.9 ± 0.8	98%
	RSD% (n = 3)	0.26	-
Urine spiked ($15\ \mu g\ l^{-1}$)	Native concentration	ND	-
	Determined concentration	14.2 ± 0.3	94%
	RSD% (n = 3)	0.61	-

[a]ND: not detected.

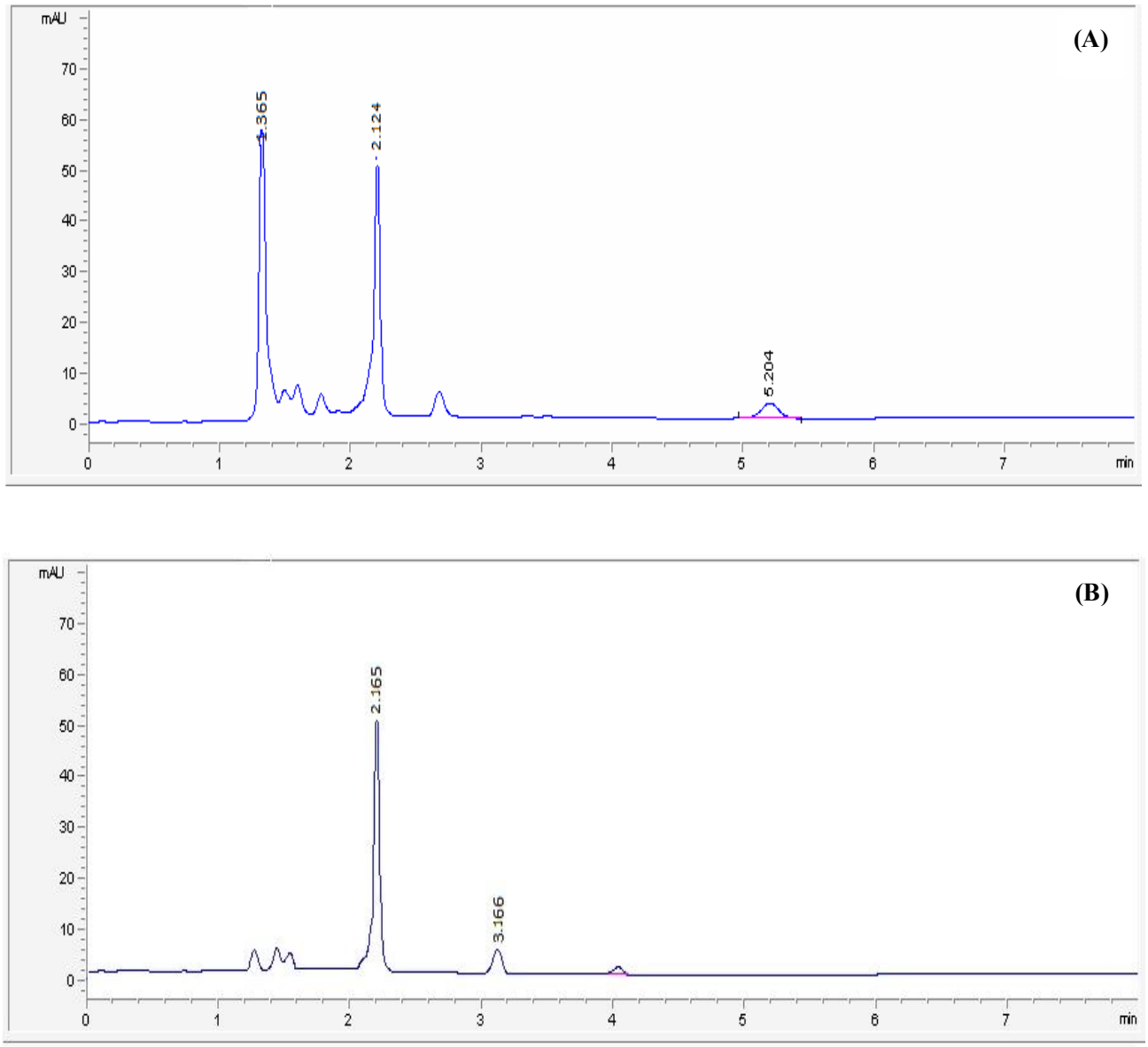

Fig. 7. The Metoprolol Succinate drug chromatogram of (A) urine sample spike 15 µg l^{-1} and (B) water sample spike 5 µg l^{-1}.

further increase the rate goes up to 1100 rpm, the efficiency is not significantly affected thus the 800 rpm was selected as the optimum revolution speed for the next experiments.

Extraction time. The time of extraction is another parameter that can affect the pre-concentration efficiency [38]. In this research, the Metoprolol Succinate drug pre-concentration efficiency is considered as a function of time in the range of 10-100 min. As can be seen in Fig. 6, by increasing the time of extraction, the drug pre-concentration is increased up to 65% in 50 min. Thus, 50 min is selected as the optimized time for the further extraction procedure.

Analytical Method Validation

The proposed method based on HF-LPME was

validated with different analytical parameters including detection limit (LOD), quantification limit (LOQ), linear dynamic range (LDR) and precision. These parameters were calculated under matrix matched calibration by varying the concentration from 15 to100 μg l^{-1} of drug free urine sample and 5 to100 μg l^{-1} of drug free tap water sample to get a proper LDR. For urine sample, the obtained LOD (3 × S/N, n = 3) and LOQ (10 × S/N, n = 3) were 3.90 and 13.02 μg l^{-1}, respectively, with the high detection coefficient (R^2 = 0.995). For water sample, the obtained LOD and LOQ were 1.5 and 5.0 μg l^{-1}, respectively, with R^2 = 0.998. The results imply that this method can measure the target compound concentration in μg l^{-1} level. After achieving the low LOQ and high pre-concentration factor of 192 for tap water and 169 times for urine samples, the repeatability and reproducibility of the proposed method were conducted based on the intraday and interday principals for three extractions in a day, and nine extractions in different days for the both samples. Hence, appropriate precision (RSDs %) was obtained for repeatability (1.3-2.7%, n = 3) and reproducibility (3.4-6.2%, n = 9).

Analysis of Real Samples

To evaluate the efficiency of HF-LPME method in field application, Metoprolol Succinate drug extraction from real samples of urine and tap water were performed. The samples were spiked with the adequate amount of the drug (15 μg l^{-1} in urine and 5 μg l^{-1} in tap water) and extraction procedure was performed. The extraction recovery and the RSD% for the proposed samples are listed in Table 1 under the optimum condition. The HPLC-UV chromatogram for the spiked and unspiked samples are shown in Fig. 7.

CONCLUSIONS

The obtained results in present research showed an appropriate method to extract the Metoprolol Succinate drug from water and urine samples with a three-phase method based on porous hollow fibers. The pH gradient was found to be as a highly efficient and a favorable parameter to transfer the analyte from SP to RP. The results of extraction from real samples showed that this method has a high selectivity and sensitivity. The recommended method has a proper precision, selectivity and detection limit, and it is noteworthy due to minimizing the organic solvents consumption. Moreover, based on the non-noisy chromatograms, it can be claimed that the hollow fiber liquid phase micro extraction is a favorable method to extract the analytes from the complex matrix of biological samples.

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