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Hollow-Fiber liquid-phase Microextraction Followed by High Performance Liquid Chromatography for the Determination of Trace Amounts of Methylphenidate Hydrochloride in Biological Fluids

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ABSTRACT

Methylphenidate hydrochloride is used fortreatment ofhyperactivity disorder. In the current work, for the first time a microextraction technique was introduced to detection and quantification of methylphenidate hydrochloride in urine and plasma samples. Hollow ûber based liquid phase microextraction (HF-LPME) followed by high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection was used for extraction of methylphenidate hydrochloride. The organic membrane solventconsists of 1-Octanol immobilized in the pores of a hollow fiber. A pH gradient was driving force to migrate analyte from sample solution, through the organic liquid membrane into an acidic acceptor solution which was located inside the lumen of hollow fiber.Extraction recoveries upper than 80% were obtainedin different biological matrices which resulted inpreconcentration factors upper than 112 and acceptable repeatability (2.4< RSD% <4.8). The method offers good linearity with estimation of coefficient higher than 0.9990. Finally, it was applied to the determination and quantification of methylphenidate hydrochloride in biological samples.

Key words: Methylphenidate hydrochloride; High performance liquid chromatography; Hollow fiber based liquid phase microextraction; Microextraction.

INTRODUCTION

Methylphenidate hydrochloride is a medicine which is used in attention-deficit hyperactivity disorder[1]. The chemical structure and phisico-chemical properties of the drug are tabulated in Table 1[2]. Methylphenidate hydrochloride is used as part of a program for the treatment of people who have attention-deficit hyperactivity disorder. Methylphenidate hydrochloride works by affecting certain chemicals in the brain, which may help to reduce some of the symptoms of attention-deficit hyperactivity disorder. Methylphenidate hydrochloride is not suitable for everyone and some people should never use it. Other people should only use it with special care. It is important that the person prescribing this medicine knows your full medical history[3, 4].Over time it is possible that Methylphenidate hydrochloride can become unsuitable for some people. Therefore, it is necessary to presenta new method that improves detection and measurement of Methylphenidate hydrochloride in biological fluid samples in order to identify thoseatrisk[5,6].

Severalmethodshave been presented in order to detection and quantification ofMethylphenidate hydrochloride up to now. There are some methods for detection and quantification of Methylphenidate hydrochlorideconcentration in biological samples includingreverse phase high performance liquid chromatography (RP-HPLC)[7, 8], liquid chromatography-mass spectroscopy[9, 10], Liquid chromatography– tandem mass spectrometry[11, 12],HPLC with chemiluminescence detection[13], gas chromatography-mass spectroscopy[14, 15] and gas chromatography with electron capture detection[16, 17].

Sample preparation steps should be used for determination of the drug in biological samples in all of these methods. It is difficult to obtain low detection limits without sample preparation steps. **We believe** that microextraction technique has not been reported for extraction and preconcentration of Methylphenidate hydrochloride from body fluids. In this work, for the first time, three phasehollow ûber based liquid phase microextraction (HF-LPME) followed by HPLC with ultraviolet (UV) detection was used and validated for detection of Methylphenidate hydrochloride in biological samples.

HF-LPME as one of LPME was introduced for the first time by Pedersen-Bjergaard[18, 19]. In HF-LPME technique, polypropylene porous hollow fiber membrane is used as the organic solvent carrier, in which target analyts transfer across organic liquid membrane from sample solution to acceptor phase. HF-LPME can provide better stability and sample clean-up ability than other LPME methods. HF-LPME divided into two-phase HF-LPME and three-phase HF-LPME. The extraction vial is filled with the sample solution. A Measured piece of a porous HF may be either a rod with a sealed bottom or a u-shape where both ends are connected to guiding tubes. Before extraction, the HF is first dipped in the organic solvent for a few times to immobilize solvent in the pores, and excess solvent is removed.

The acceptor solution fills the lumen of the HF. This acceptor solution can be an organic solvent in which the same as that used for the organic solvent in HF pores, resulting in a two-phase LPME, or the acceptor solution may be an acidic or basic aqueous solution, resulting in a three-phase LPME. In the two-phase LPME, the target analytes are extracted from the aqueous sample and into the organic solvent (acceptor solution) present both in the porous wall and inside the lumen of the HF[20-24]. In three-phase LPME, the analytes are extracted from the aqueous sample, through the organic solvent in HF pores, and further into the aqueous acceptor solution present inside the lumen of the HF[25-37].

In this work, the effects of various variables on HF-LPME efficiency were investigated and optimized. After optimization, the method followed by HPLC-UV was applied for extraction and determination of Methylphenidate hydrochloride in plasma and urine sample as biological samples.

EXPERIMENTAL

Chemicals and materials

All chemicals were of analytical-reagent grades and used as received. Methylphenidate hydrochloridestandard were kindly donated byDrug and Food Administration (Tehran, Iran).1-Octanol, dodecane, n-decane, n-hexane, were purchased from Merck (Darmstadt, Germany).Acetonitrile and methanol of HPLC were obtained from Merck (Darmstadt, Germany). Sodium hydroxide and sodium chloride were obtained from Sigma–Aldrich (St. Louis, MO, USA). Distilled water was deionized by a Milli-Q water purification system from Millipore (Madrid, Spain).

The PPQ3/2 polypropylene hollow fiber (600 μm ID, 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana GmbH (Obernburg, Germany) and used as received.
Stock solutions of analyte of about 1000 mg L^{-1} were precisely prepared in methanol. They were all stored in the darkness at 4 ÚC and working analyte mixtures were daily prepared by dilution with the appropriate volume of distilled water.

Apparatus and software

Separation and detection of the target analyte were carried out by a Younglin YL9100 HPLC (Seoul, Korea) equipped with a Quaternary9110 HPLC pump (Korea), a 4-channel

mixing valve with a 10 µL sample loop, YL9101 vacuum degasser and a YL 9120 UV-Vis detector. Chromatography data were recorded and analyzed using Younglin Auto Chro 3000 software.The separations were performed on an ODS-3 column (250 mm × 4.6 mm, with particle size of 5 µm) from MZ-Analysentechnik (Mainz, Germany).The mobile phase consisted of 50 mMpotassium dihydrogen orthophosphate and methanol mixture (57:43), under isocratic condition. The flow rate of the mobile phase was set at 1.0 mL min^{-1} andtotal analysis time was 15 min.The injection volume was 10 µL for all of the samples and detection was performed at a wavelength of 210 nm.

HF-LPME procedure

Ten mL of sample solution was ûlled into a 15 mL vial. Extraction process was shown in fig. 1.A 5.0 cm piece of fresh ûber was inserted into the needle tip of a 25 µL Hamilton syringe that was previously ûlled with acceptor phase. Subsequently, the fiber dipped for a 10 s period into the organic solvent. After filling hollow fiber wall pores with organic membrane solvent, excess amount of organic solvent washed with distilled water, and 10 µL of acceptor solution with pH=1.9 was injected into the lumen of hollow ûber with the Hamilton syringe, and the lower end of the hollow fiber was mechanically sealed by a piece of foil. Subsequently, the ûber was placed in the sample solution vial. Extraction vial was placed on a magnetic stirrer plate to provide effective stirring condition during the extractions. During extraction, the solution was stirred at 750 rpm. After extraction, the acceptor solution was collected into a micro-vial by Hamilton syringe.Finally, acceptor solution was injected for analysis into the HPLC instrument.

Real sample analysis

Drug-free human plasma was kindly denoted by Iranian Blood Transfusion Organization (Tehran, Iran). Urine samples were obtainedfrom healthy young volunteer. The samples were stored at “4°C, thawed and shaken before extraction.

Calculation of preconcentration factor, extraction recovery and relative recovery

The preconcentration factor (PF) was defined as the ratio of the final analyte concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte ($C_{i,s}$) in the sample solution:

$$PF = \frac{C_{f,a}}{C_{i,s}} \quad ...(1)$$

where $C_{f,a}$ was calculated from a calibration graph obtained by direct injection of analytes standard solutions (0.2-200 mg L^{-1}) in 10 mMHCl. Extraction recovery (ER) was defined as the percentage of the number of moles of analyte which was extracted to the acceptor phase ($n_{f,a}$) divided by the number of moles of analyte originally presented in the sample solution ($n_{i,s}$).

$$ER = \frac{n_{f,a}}{n_{i,s}} \times 100 = \frac{C_{f,a} \times V_{f,a}}{C_{i,s} \times V_{i,s}} \times 100 \quad ...(2)$$

$$ER = \left(\frac{V_{f,a}}{V_{i,s}}\right) PF \times 100 \quad ...(3)$$

where$V_{f,a}$ and $V_{i,s}$ are the volumes of acceptor phase and sample solution, respectively. Relative recovery (RR) was calculated by the following equation:

$$RR = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad ...(4)$$

whereC_{found}, C_{real}, and C_{added} are the concentrations (µg L^{-1}) of analyte after addition of known amount of standard into the real sample, the concentration of analyte in real sample, and the concentration of known amount of standard which was spiked into the real sample, respectively.

RESULTS AND DISCUSSION

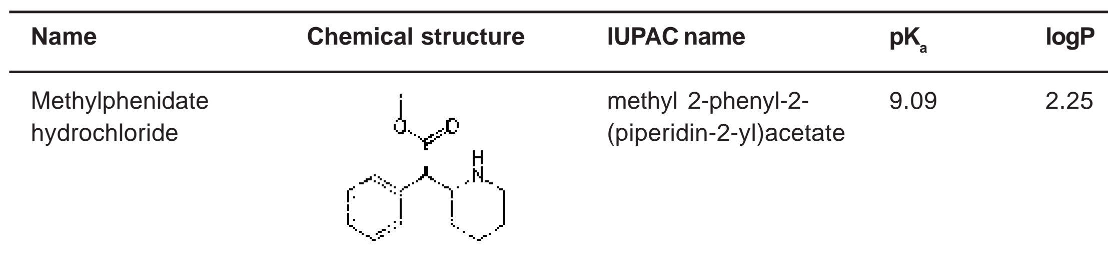
In order to obtain the maximum extraction efficiency for preconcentration and determination of Methylphenidate hydrochloride in biological fluids, the major parameters on HF-LPME, including, organic membrane solvent, sample solution stirring rate, extraction time, pH in donor and acceptor phases, and temperature were investigated and optimized by one variable at the time method.. All optimizations steps were performed in ultra-pure water.

Organic membrane solvent

In order to obtain ideal extraction efficiency

in HF-LPME, selection of an organic membrane solvent is necessary. The organic solvent forms a thin layer within the wall of the hollow fiber. The extraction organic solvent must be compatible with the hollow fiber so that the pores in the wall of the hollow fiber can be ûlled completely. In addition,

Table 1: Chemical structures, pK_a and logP of methylphenidate hydrochloride.



Name	Chemical structure	IUPAC name	pK_a	logP
Methylphenidate hydrochloride		methyl 2-phenyl-2-(piperidin-2-yl)acetate	9.09	2.25

Reference [2]

Table 2: Figures of merit of HF-LPME in drug-free distilled water sample

LOD	(ngmL-1)	LOQ (ngmL-1)	Linearity (ngmL-1)	R^2	PF[a] Within day	RSD%[b] Between day
3.0	12.0	12.0-5000.0	0.9990	112	2.5	3.5

[a] Drugs were present at 500 ng mL^{-1}.[b] Within day and between day RSDs% were obtained by four replications.

Table 3: Determination of methylphenidate hydrochloride in different urine and plasma samples

Sample	C_{real} ($ngmL^{-1}$)	C_{added} (ng mL^{-1})	C_{found}(ng mL^{-1})	RSD% (n = 3)	RR%
Plasma 1	nd[a]	0.2	0.82	2.4	82
Plasma 2	nd0.5	1.8	2.6	90	
Plasma 3	nd1.0	4.0	3.2	80	
Urine 1	nd0.2	0.17	3.2	85	
Urine 2	nd0.5	0.84	3.6	84	
Urine 3	nd1.0	1.6	4.3	80	
Urine 4	nd2.0	4.3	4.8	86	

[a] Not detected

Table 4: Comparison of the HF-LPME with other analytical techniques for determination of methylphenidate hydrochloride

Analytical method	Sample preparation method	Sample	LOD (ng L-1)	Linearity (ng L-1)	RSD%	Ref.
HPLC	HF-LPME	Plasma/urine	3	12-5000	3.0	This work
HPLC	LLE	plasma	1	1.0-80.0	7.5	[40]
GC-MS	SPME	Oral fluids	1	2-256	15	[41]
HPLC	LLE	urine	2-8	10 -3000	6.2-7.8	[42]

organic membrane solvent should not be miscible in sample and acceptor solution and should have good affinity for the target analyte in order to extraction target analyte from sample solution to acceptor phase[38]. Therefore,1-Octanol, n-dodecane, n-decane, and n-hexane were investigated as organic membrane solvent. As shown in Fig. 2A,1-octanol showed the higher extraction efficiency than the others for Methylphenidate hydrochloride. Therefore, 1-octanol was selected as optimal organic membrane solvent.

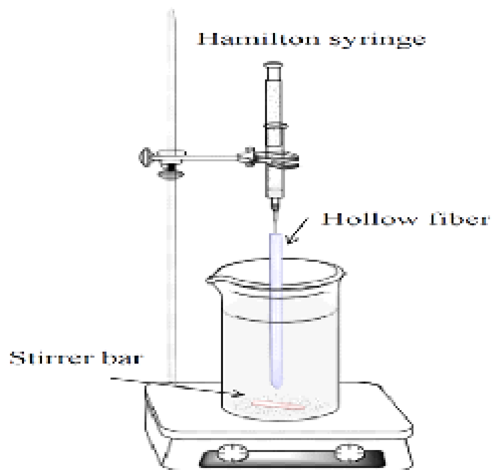


Fig. 1: Schematic diagram of proposed HF-LPME setup

Effect of the pH in donor and acceptor phase

To obtain best extraction efficiency in HF-LPME for target analyte, the donor phase and acceptor phase pH adjustment are necessary. Donor sample solution should be adjusted to a pH where the target analyteis uncharged, because uncharged molecules have a good affinity to organicmembrane solvent. The acceptor solution pH has a pH where the analyte is charged in order toprevent them back extraction into the organic membrane solvent[39]. For this reason, donor and acceptor phase pH should be adjusted two or three units under and over pKa values, respectively.The acidity constant (pKa) of methylphenidate

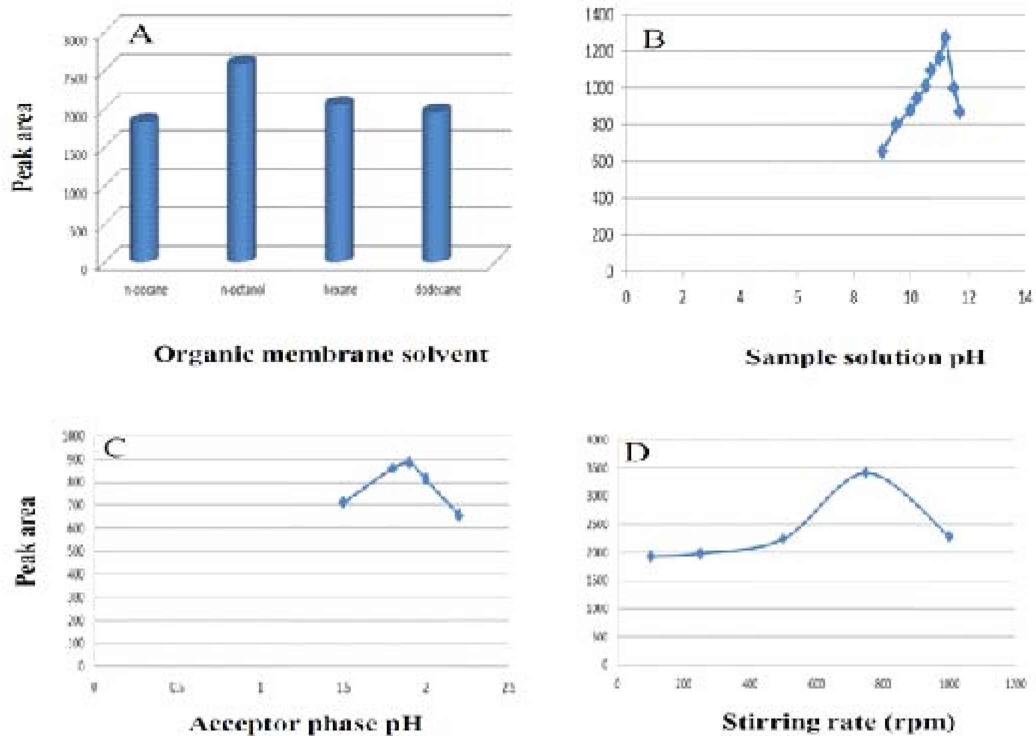


Fig. 2: Optimization of (A) organic membrane solvent, (B) donor sample solution pH, (C) acceptor phase pH and (D) stirring rate for extraction of methylphenidate hydrochloride

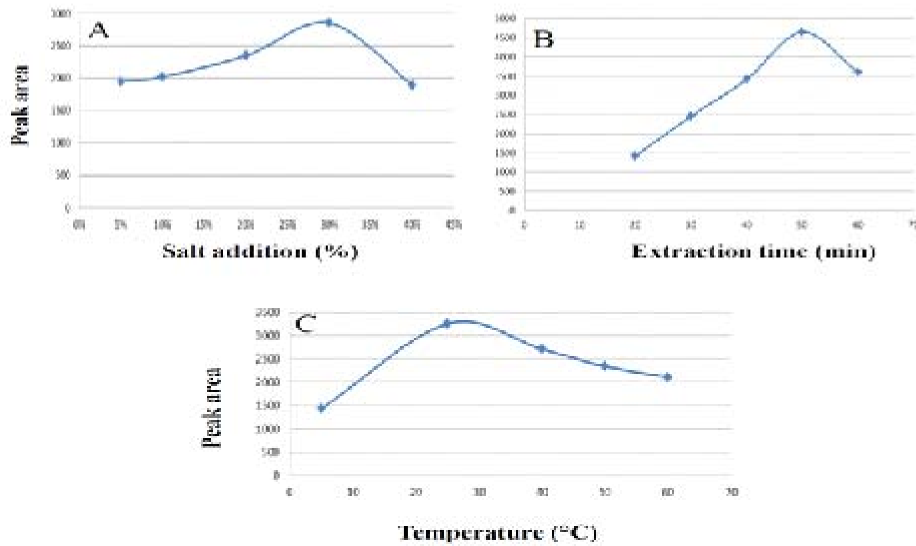


Fig. 3: Optimization of (A) salt addition effect, (B) extraction time and (C) temperature for extraction of methylphenidate hydrochloride

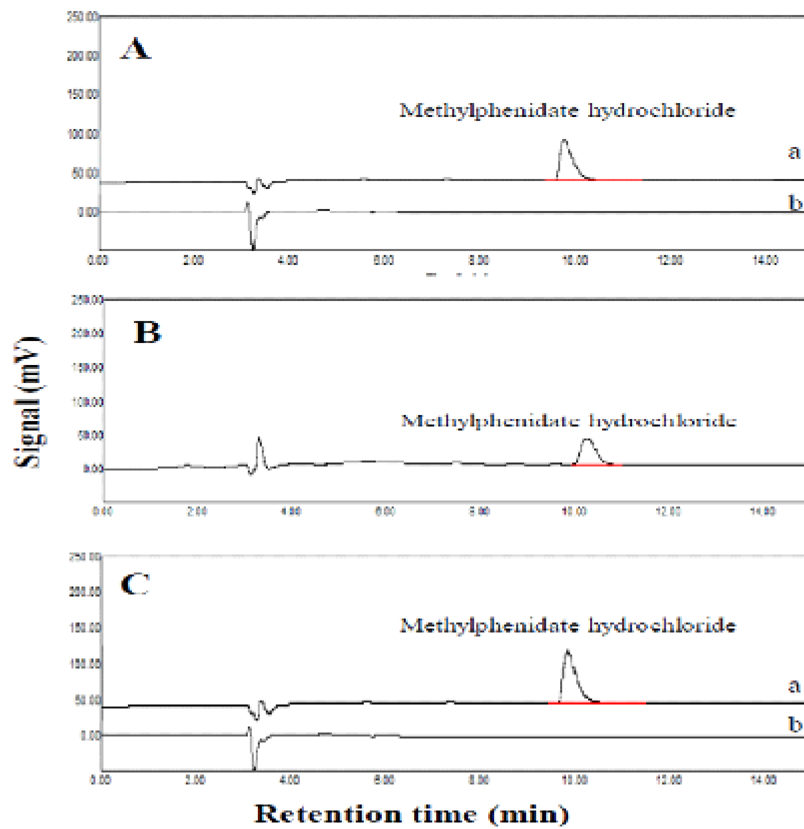


Fig. 4: Chromatograms obtained after HF-LPME extraction of (A) urinesample, (B) patient that used methylphenidate hydrochloride tablet and (C) plasma sample ((a) spiked sample and (b) non-spiked sample at a concentration level of 1.0 mg L^{-1})

hydrochloride being around 9.78 values. For this purpose, donor and acceptor phase pH was investigated in pH from 9 to 11.7 and 1.5 to 2.2, respectively. As shown in fig. 2B and 2C, the highest extraction efficiency was obtained using pH=11.2 and pH=1.9for Methylphenidate hydrochloride. Therefore, pH=11.2 for donor phase and pH=1.9 for acceptor phasewere used for following experiments.

Effect of stirring speed

Stirringof sample solution have greet effect to the analyte diffusion, which can be acceleratedextraction process and shorten the time of extraction to reach the extraction equilibrium.Stirring of sample solution facilitates analyte diffusion from donor phase into the acceptor phase[38].In the present work, the effect of the stirring speed from 100 to 1000 rpm onthe extraction efficiency was studied. As shown in fig. 2D, when the stirring speed was increased from 250 to 750 rpm, the extraction efficiency oftarget analytewas increased. However, increasing in stirring rate over 750 rpm, decrease extraction efficiency due to bobble formation around the hollow fiber in extraction process. Hence,thestirring rate of 750 rpm was chosen as the optimal stirring speed for following experiment.

Salting-out effect

The salting-out influence is widely used to increase the extraction recovery of uncharged target compounds from aqueous sample. It consists in decreasing the solubility of uncharged compounds in donor phase by increasing the ionic strength. In present work, the effect of various concentrations of NaCl from 0 to 40 percent to extraction of target analytewas tested. Theextraction efficiency enhanced when the NaCl concentration was increased from 0 to 30% (w/v). Over 30% salt addition decreased extraction efficiency. As shown in fig. 3A, 30% salt addition showed high extraction efficiency, and used in the future experiments.

Extraction time effect

The extraction time is amajor parameter in HF-LPME technique.HF-LPME like other miniaturized sample preparation techniques such as SPME; several minutes to hours take to reach an equilibrium that ensures optimal extraction efficiency. In this work, efficiency of methylphenidate hydrochloride extraction was studied in the range of 20–60 min. Fig. 3Billustratethe result ofdifferent extraction time on extraction efficiency. The extraction efficiency increased by the increasing of the extraction time up to 50 min. Future increasing in the extraction time to 60 min, decreasing the extraction efficiency due to instability in organic membrane solvent. Therefore, 50 min was selected as the optimal extraction time in the future experiments.

Influence of extraction temperature

The temperature of extraction has to be investigated because this parameter cans influence the partition coefficient of the analyte between the various phases. To study the effect of the temperature, the extraction vial was placed in an oil bath to heat the donor phase from 5 ÚC to 60 ÚC. The results, presented in Fig. 3C show that a temperature enhancement increased transfer of target analyte to the acceptor phase until 25 °C. A strong decrease of the extraction efficiency was observed by more increasing extraction temperature over 25 °C. This can be due to increasing the miscibility of 1-octanol in water at high temperature or to the partial evaporation of this organic solvent. In conclusion, a temperature of 25 °C was chosen as optimal extraction temperature.

As a consequence, the optimal conditions were attained by using 11.2 and 1.9 as donor and acceptor phases'pH, respectively, and using 750rpm as stirring speed for 50 min. In addition, the organic membrane composition was 1-Octanol. Thirtypercent salt addition and of 25 °C assample temperature was selected as best condition for Methylphenidate hydrochloride extraction.

Method performance

To evaluate the practical applicability of the proposed HF-LPME method, figures of merit were investigated using standard solutions of the analyte in a drug-free urine and plasma samples. Optimal condition was applied to find out linearity, repeatability, and LODs of this method that summarized inTable 2.Under the optimized conditions the calibration curve was linear in the

range of 12–5000 μg L^{-1} with coefûcient of determination (r^2)more than 0.9990. The relative standard deviations (RSD %) for extraction of the analytewere less than 2.5% and 3.5% for intraday and interday experiment, respectively. LODs less than 3.0 μg L^{-1} wasviewed for target analyte. PF values higher than 112-fold were obtained for the extraction of methylphenidate hydrochlorideby comparison slope of calibration curve before and after extraction process.

Analysis of real sample

HF-LPME is a powerful method for isolation and cleanup of target analyte from untreated biological fluids. Thus, the optimal conditions of HF-LPME were used for extraction of the target analytefrom human plasma and urine samples. To reduce matrix effects calibration curves were plotted in drug free urine and plasma samples.

Extraction from human urine sample

Drug-free human urine was spiked with proper amount of the target drug and extraction was accomplished after dilution of urine samples (1:3) and addition of proper amount of NaOH solution to achieve pH 11.2. The results are summarized in Table3. RSD% values less than 4.8% confirm the acceptable precision of proposed HF-LPME method. To evaluate the applicability of HF-LPME for human urine, four urine samples were analyzed with the proposed method. Since no methylphenidate hydrochloride was found in samples, all urine samples were spiked with the target drug at a different concentration level. Chromatograms are shown in Fig. 4A. To investigate the capability and accuracy of the proposed HF-LPME method, a urine sample was collected from a volunteer used 10 mg methylphenidate hydrochloride tablet, after 12 h of the last use. Figs. 4B show the typical chromatograms of real urine sample that collected from a patient that used methylphenidate hydrochloride tablet.

Extraction from human plasma sample

Plasma sample was diluted with water (1:3) and adjusted to pH 11.2 by addition of proper amount of NaOH solution. The drug was spiked into the human plasma and their quantitative analysis was evaluated under optimized conditions. Precision of the method was determined by three-replicate extraction of the drugs from samples at different concentration level. The RSD% was found less than 3.2% for Methylphenidate hydrochloride. To evaluate the applicability of HF-LPME for human plasma, three plasma samples were analyzed with the proposed method. Since no methylphenidate hydrochloride was found in samples, all plasma samples were spiked with the target drug at a different concentration level that showed in Table 3. Chromatograms are shown in Fig. 4C.

Comparison of the proposed method with other techniques

The present method was compared with the other methods in terms of validation and precision. As can be seen, the method is quite comparable to those mentioned in Table 4.

CONCLUSIONS

The present study exhibited an excellent performance of the HF-LPME technique for the extraction of methylphenidate hydrochloride drug from biological fluids. Up to 112-fold enrichment factor and effective sample clean-up were obtained. Accordingly, it is concluded that HF-LPME is an effective method to preconcentration of methylphenidate hydrochloride drug from the biological samples prior to HPLC analysis. The results indicated that hollow ûber microextraction method has an excellent cleanup, high enrichment, factor and can be served as a simple and sensitive method for monitoring of methylphenidate hydrochloride drug in the biological samples.

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