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Preparation of Molecularly Imprinted Polymers Coupled with Magnetic Nanoparticles for the Selective Extraction of Propranolol from Aqueous Solution of Propranolol, Atenolol and Metoprolol

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Abstract

In this work, highly selective molecularly imprinted polymers (MIPs) of propranolol on the internal surface of hollow fiber were prepared. The resulting MIP coated MNP were used as dispersive solid phase micro extraction (SPME) fiber coupled with HPLC-UV for the selective extraction and detection of propranolol. Encouraging result was obtained. Method was evaluated and enrichment factors, relative standard deviations and linearity ranged for propranolol 918.8, 2.9% and 25-3000 ng.ml-1 were obtained, respectively.

Keywords: Molecularly Imprinted Polymers, HPLC-UV, Propranolol.

Introduction

Propranolol, a non- selective β-blocker used in the treatment of hypertension, angina pectoris and cardiac arrthmias, (Feely, 1994) has the structural formula shown in fig 1.

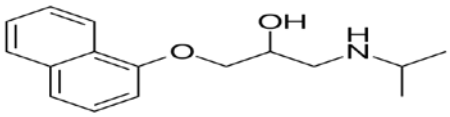

Fig. 1: Propranolol, mol. Wt = 259.34

The methods have been developed for determination of propranolol, including spectrophotometric methods (El-Saharty, 2003) and colorometric methods (Sultan, 1988; ElSayed et al., 1989; Martiínez et al., 2000). Many chromatographic procedures are described for the assay of propranolol. These include TLC (Ruane and Wilson, 1988), GC (Black et al., 1996), HPLC (Walshe et al., 1996; Bergmann et al., 1995; ChuongPham et al., 1995; Egginger et al., 1994) and capillary electrophoresis (Zelikman and Hjerten, 1989). The methods available in the literature for the simultaneous determination of these drugs are either densitometry (Geissler and Mutschler, 1978), or laborious HPLC (Cline-Love and Fett, 1991; Walshe et al., 1996).

Nanomaterials have unique properties for commercial applications (Mahmoud, et al. 2018). Nanoparticles offer distinct properties such as particle size, increased chemical reactivity, and increased surface area/mass ratio compared to their bulk counterparts (Haripriya & Ajitha, 2017). In this study, MIP improved with magnetic nanoparticles by micro syringe injected in to the hollow fiber.

Molecular imprinting is an increasing applied technique to build selective recognition sites in a stable polymer matrix. MIPs have prearranged structure and specific and enrichment of analytes (Wang et al.,2011; Ma et al., 2011; Zhou et al., 2010; Zhang et al., 2010; Gai et al., 2010; Wang et al., 2009; Li et al., 2009). MIPs have wide applications in chromatographic separation, sensors (Holthoff and Bright, 2007), SPME (Mullett et al., 2001), DSPE (Wang et al., 2011; Ma et al., 2011), and other fields (Paiket al., 2009; Balogh et al., 2010). Experimental results demonstrated that MIP coated MNPs are selective toward the target molecules and achieve a rapid separation and enrichment because of the efficient magnetic separation.

Finally, we used MIP-coated MNPs coupled with HPLC-UV for the selective monitoring of trace propranolol. According to our research, this article was the first attempt to synthesize the magnetic molecularly imprinted nanoparticles for recognition of propranolol.

Experimental

Materials

Propranolol, atenolol and metoprolol were purchased from Samisaz pharmaceutical co., ltd. The methanol, water and acetonitrile of HPLC grade were obtained from Merck (Darmstadt, Germany). Methacrylic acid (MAA), azo (bis)-

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isobutyronitrile (AIBN) and Ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma–Aldrich.

Instrumentation

An14163-D HPLC system (Knauer, Germany) comprising S1000 pump, S2600 ultraviolet detector. A Knauer C18 column (250mm×4.60mm, 5µm) was used for above chromatographic separations. Ultraviolet detector was set at 220 nm for β-blockers detection. The mobile phase consisted of water/ methanol/ acetonitrile (50:25:25 v/v) at a flow rate of 1.0 mL.min^{-1}. The injection volume was 20 µL. Other instrument used included Metrohm (Switzerland) pH meter, ED224S balance (Sarturius Co, Germany), C -MAG HS 7 magnetic mixer (IKA Processing Equipment, Germany).

Synthesis of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles are prepared by co-precipitation method. This process is done in two stages. In the first stages, dissolved 5.2 g $FeCl_3$. $6H_2O$ and 2 g $FeCl_2$. $4H_2O$ in 25 ml 0.4 M HCL under N_2 gas for 30 min. In the other solution, 250 ml 1.5 M NaOH was mixed with stirring and heated at 80°C under N_2 for 1 h. Then the first solution was added slowly to the second solution. The product was collected by an external magnetic field and dried in a vacuum.

To obtain the mono dispersing nanoparticles, the nanoparticles were improved with trisodium citrate. Fe_3O_4 nanoparticles washed with acid (2 M) for 5 min.

Preparation of MIP coupled with NMP coated SPME fiber.
The silica fibers (about 2 cm length) were cleaned with ethanol. Subsequently, the fibers were washed with water and soaked in 1 mol l-1 Hydrochloric acid for 1 h at room temperature. Fibers were washed with water again and dried at room temperature.

MIPs were prepared by 3.5 mg of propranolol and 50 µl of MAA dissolved in 3 ml of methanol. Pre- polymer solution was stored for 12h, and then 0.5 ml EGDMA, 4.6 mg AIBN and 0.03 g Fe3O4 nanoparticles were added and adequately dissolved. Solutions for the non-imprinted polymer (NIP) were prepared in the same way as those for the MIP but without the propranolol. The solution was poured into a small tube and deoxygenized with a stream of nitrogen for 5 min. The solution injected into the hollow fiber and then the fiber ends were blocked. Polymerization was performed for 12 h at 60°C. The fibers with new coating on internal surface were obtained by pulling it out of the oven.

SPME method and chromatographic analysis.

At first to remove the propranolol template, fibers soaked in 5 ml of 10% acetic acid in methanol for 30 min. this method was performed repetitiously until the propranolol could not be detected in solution by HPLC.

The fiber was immersed in aqueous solution of propranolol for extraction for 30 min at the stirring of 500 rpm and then outer surface of fiber was washed with water for 1 min. subsequently, in order to return extraction process fiber was immersed in 0.3 ml methanol (optimize desorption solution) for 30 min at the stirring of 500 rpm. The sample was transferred to the desorption solvent and injected into the chromatographic column for analysis. A Knauer C18 column (250mm×4.60mm, 5µm) was used as the LC column. The mobile phase was acetonitrile/ methanole/water (25:25:50 v/v) at the flow rate of 1.0 ml min^{-1}. Determination wavelength was 228 nm for propranolol.

Results and Discussion

Optimization of initial extraction time (T1) and desorption time (T2)

Experimental results on adsorption kinetics for 1 ppm propranolol as analyze are demonstrated in fig. 2. The extraction yield increases quickly along with increment of extraction time within 30 min, and adsorption reaches equilibrium at 30 min. the reason may be the specific affinity of MIP coating to propranolol molecule. Loose and porous structure of MIP coating is another contributing factor, by which diffusion speed can be increased for the analyses into or out of coating. The equilibrium time of desorption for 1 ppm propranolol is about 40 min.

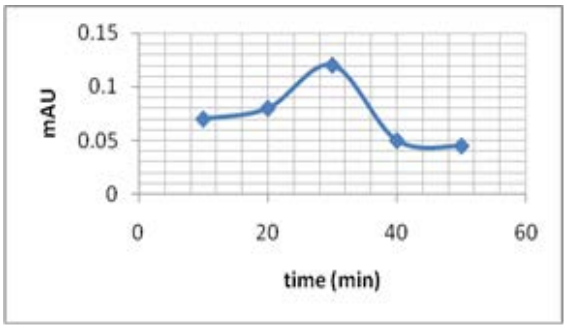

Fig. 2: Optimizing the initial extraction time (T1).

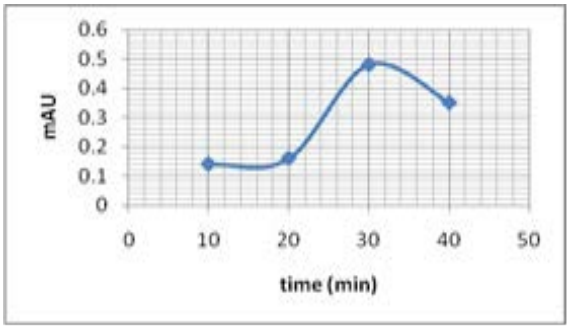

Fig. 3: Optimizing desorption time (T2).

Fiber selectivity

To investigate the selectivity of propranolol MIP coated fiber, two β-blockers (atenolol and metoprolol) were selected and their

structures are shown in fig. 4. In order to prevent saturation of the MIP-coated fiber, the extraction of propranolol, and other β-blockers were investigated at 1 ppm level.

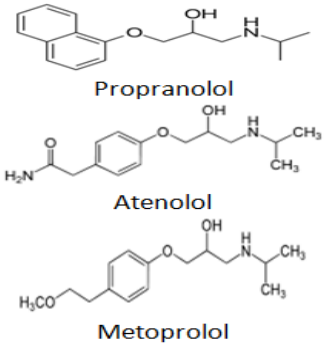

Fig. 4: Structures of propranolol, atenolol and metoprolol.

The extraction efficiencies of propranolol, atenolol and metoprolol to MIP and NIP –coated fibers are in shown in Fig. 5.

Effect of pH

PH is one of the important factors in the process of propranolol drug adsorption on fibers and finally increasing the efficiency of micro-extraction. The propranolol pKa value is 9.4. Therfore these compositions have natural form in an environment with a pH lower than their pKa, and their tendency would be increased to be absorbed in fiber. We selected different values of pH from 4 to 7 to obtain pH (fig 6). The highest extraction efficiency was observed in pH=5/8.So this pH was selected as optimal one, and all measurements were performed at this pH.

The effect of desorbed organic solvent

Selecting the type of solvent is one of the factors affecting micro extraction process. Fore organic solvents such as methanol, ethanol, 2-propanol and acetonitrile were studied as desorption solvents. The results obtained have been shown in Fig. (7).

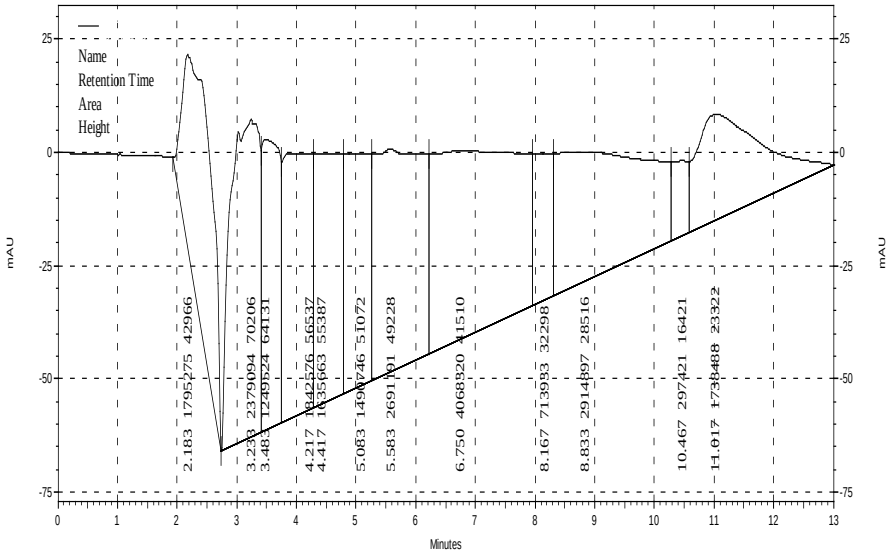

Fig. 5: Chromatogram of propranolol in optimal condition

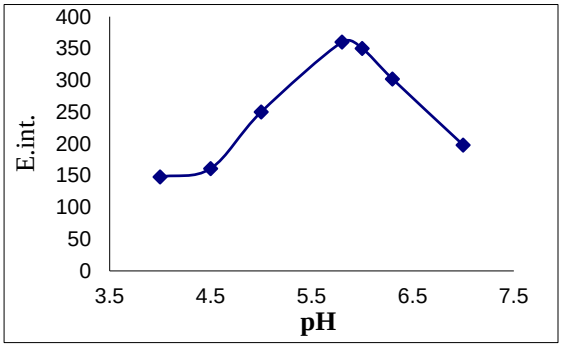

Fig. 6: Effect of PH for aqueous phase. pH:5/8, aqueous phase volume:12 ml, stirring rate: 500 rpm, extraction time: 30 min, desorbed solvent volume: 0.7 ml, desorption time: 30 min, desorption organic solvent: methanol.

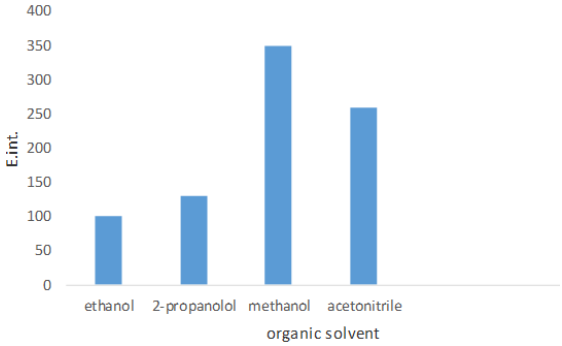

Fig. 7: The effect of desorbed organic solvent. aqueous phase volume:10 ml, stirring rate: 500 rpm, extraction time: 30 min, desorbed solvent volume: 0.7 ml, desorption time: 30 min, desorption organic solvent: methanol.

The effect of aqueous phase volume

In solid phase micro-extraction, the analyses are extracted from high volume of aqueous phase to very low volume of receiving phase. In this project, the receptor phase volume was kept fixed and the donor phase volume was changed, and its effect was studied in extraction efficiency (Fig. 8). The results showed that the maximum extraction efficiency is observed in volume of 12 ml of aqueous phase. Therefore, all measurements were performed on 12 ml of aqueous sample.

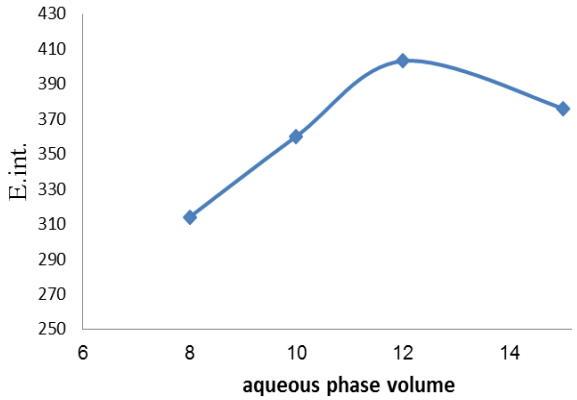

Fig. 8: The effect of aqueous phase volume. phase volume:10 ml, stirring rate: 400 rpm, extraction time: 30 min, desorbed solvent volume: 0.7 ml, desorption time: 30 min.

The effect of stirring rate

Stirring the solution causes increase in mass transfer rate, and reduces the time to reach thermodynamic equilibrium. So it seems that vigorous stirring increases the extraction efficiency. The sol - gel absorbent is inside the hollow fiber, so the solution vigorous stirring appears logical. Various stirring rates from 200 to 800 rpm were studied in this project, and the extraction efficiency was compared for each one of them (Fig. 9). The results showed increasing the stirring rate to 50 rpm leads to increase in the extraction efficiency. Decreasing the extraction efficiency at very high rates is attributed to formation of air bubbles. On the other hand, hitting the hollow fiber to magnet and the vial walls at high stirring rate caused damage to fiber and decreasing the extraction efficiency.

The important note is that the mentioned contents are also true about desorption solvent stirring and stirring up to 500 rpm increases the extraction efficiency. According to mentioned contents, the stirring rate of 500 rpm was used in all experiments of current project.

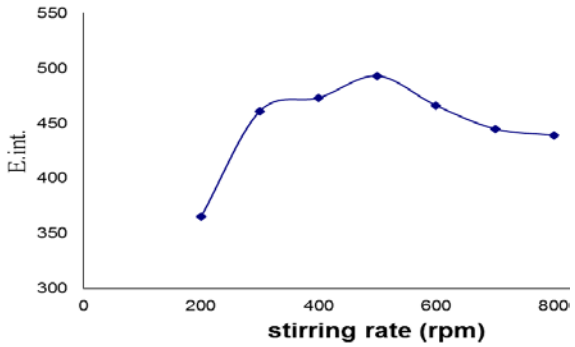

Fig. 9: The effect of stirring rate. pH:5/8, aqueous phase volume: 12 ml, stirring rate: 400 rpm, extraction time: 30 min, desorbed solvent volume: 0.7 ml, desorption time: 30 min, desorption organic solvent: methanol.

The effect of desorption solvent volume

In HF-SPME, the analyses are desorbed by a suitable volume of organic solvent. As in this project, ethanol has been introduced as a suitable desorption solvent in most cases, mainly due to the unique properties of methanol compared to other solvents. The desorbing solvent volume plays an important role in efficiency of desorbing the analyses from fiber and reaching to equilibrium. Naturally, the volume of desorbing organic solvent is less than the aqueous sample. Various volumes of 0.3-1 mL organic solvent were used in this project, and the extraction volume was compared (Fig. 10). The results showed that the maximum extraction efficiency is in volume of 0.3 mL of organic solvent. The extraction efficiency is decreased in volumes higher than organic solvent. The main reason for decreasing the efficiency with increasing the desorbing solvent volume may be attributed to analyze dilution. Considering these factors, the volume of 0.3 mL of organic solvent was used in all experiment for desorbing analyze from fiber.

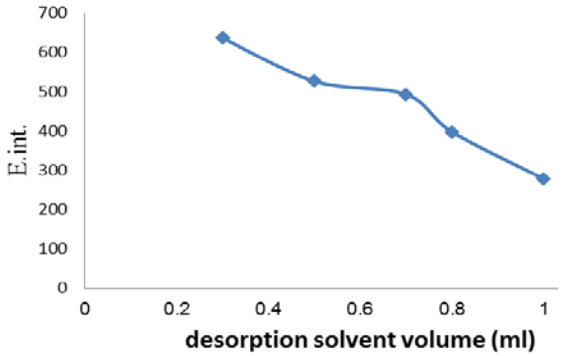

Fig. 10: The effect of desorption solvent volume. Ph: 5/8, aqueous phase volume: 12 ml, stirring rate: 500 rpm, extraction time: 30min, desorbed solvent volume: 0.7 ml, desorption time: 30 min, desorption organic solvent: methanol.

Drawing the calibration curve

It is necessary to draw a calibration diagram in quantitative analysis by spectroflorimeter. This diagram is obtained from

drawing the fluorescence emission values of standard samples according to their concentration. The appearance of correlation coefficient (R2) and calibration diagram gives a true picture of linear range situation. In order to draw the calibration curve, a set of standard solution with different concentration was prepared from propranolol. There standards are prepared under optimal condition, and measured by spectroflorimeter device after extraction and desorption.

With drawing the calibration curve in diagram (fig.11), the linear equilibrium Y= 0.0184x+12.702 with correlation coefficient of 0.9991 and linear range of 25-3000ng/mL is determined.

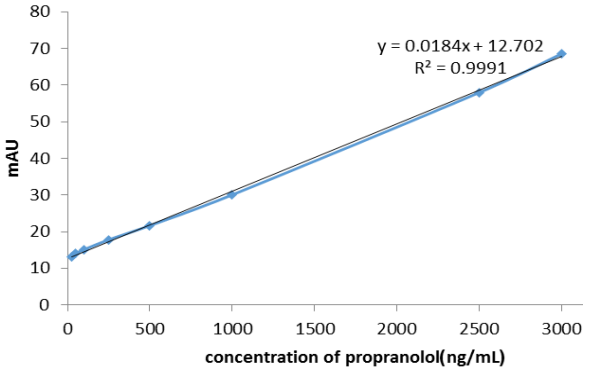

Fig. 11: The calibration curve of propranolol in optimal condition.

The study of actual sample

In this part of project, we have studied the actual sample matrix effect on extraction and separation of very low amounts of propranolol by the discussed method. Two samples of water were examined for actual sample effect. The water of well in Torghabeh area, and the water of river out of the city (Akhlamad near Mashhad). According to results obtained, no signal was observed for both water samples. In order to determine the accuracy of the method in actual samples matrix, a solution with concentration of 0/003 and 0/007 microgram per mL of propranolol was made in water samples matrix and examined. Repeatability and the percentage of analyze relative recovery in the studied actual sample matrix have been shown in table 1.

Table 1. The actual sample matrix effect

Actual sample	Added (ppm)	Founded (ppm)	Recovery %	RSD % (n=3)
Water of well	0 0.003	0 0.0029±0.0003*	---- 95.5	---- 7.3
Water of river	0 0.007	0 0.0076±0.0004*	---- 108.5	---- 5.3

*Mean ± Standard Deviation

Discussion and Conclusion

Unlike classic extraction methods, the solid phase micro-extraction is a method based on lacking the use of expensive, toxic and environment- polluting organic solvents. This method is very quick, easy and efficient for a wide variety of analyses.

In this project, a new solid phase micro-extraction absorbent was made based on MIP technique armed with functionalized carbon nanotubes. The linear range and the relative recovery obtained were also satisfactory. Totally, the conducted method is very easy, cheap and fast and has various advantages than traditional extraction methods. The discussed method is a reliable method to measure the drugs in environment and introduces the real sample.

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