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Fluorescence Detection after Solid-Phase Extraction of Atenolol from Human Serum and Pharmaceutical Waste-Water Samples by using New Magnetite Nano-Hybrid

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ABSTRACT

A magnetic solid-phase extraction (SPE) method based on polystyrene maleic anhydrid/magnetite (Fe_3O_4) Nano-hybrid as an innovative adsorbent was developed for the separation and pre-concentration of atenolol prior to its determination by spectrofluorometry. This prepared SMA/ Fe_3O_4Nano-hybrid possesses the magnetic property of Fe_3O_4 Nano-particles that makes it easily manipulated by an external magnetic field. On the other hand, the surface modification of SMA by APTES leads to selective separation of the target analyte from sample matrices. The structure and morphology of the synthesized adsorbent were characterized using powder X-ray diffraction, Fourier transform infrared spectroscopy, and field emission scanning electron microscopy. The experimental factors affecting the extraction/pre-concentration and determination of the analyte were investigated and optimized. Under the optimized experimental conditions, the calibration graph was linear in the range between 0.10 and 60 ng mL^{-1}, with a correlation coefficient of 0.9989. The limits of detection and enrichment factor for atenolol were 0.32 ng mL^{-1} and 100 ng mL^{-1}, respectively. The maximum sorption capacity of the adsorbent for atenolol was 96.7 mg g^{-1}. The method was successfully applied to monitoring atenolol in human serum and pharmaceutical waste-waters samples, with recoveries in the range of 96.0%-103.0% for the spiked samples.

Keywords: Nano-hybrid; Magnetite; Polystyrene; Magnetic solid phase extraction; Atenolol

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INTRODUCTION

In the past few years, SPE has become the most-often used pre-concentration technique for trace analysis. New types of SPE materials such as highly cross-linked polymers and chemically-modified polymers are therefore currently being developed to allow more effective extractions. In SPE with most commercial sorbents, many components of complex samples (e.g., biological, pharmaceutical and environmental samples) are co-extracted. Therefore, their determination in biological human fluid-like urine and plasma or pharmaceutical samples is vitally important. However, inspite of all the advantages of SPE, it can still be tedious, timeconsuming, and relatively expensive [1]. Recently, a new mode of SPE called magnetic solid-phase extraction (MSPE) has been developed [2]. MSPE is based on the combination of magnetic inorganic material and non-magnetic adsorbent material [3]. By taking advantage of the combined benefits of both the materials, the MSPE technology exhibits excellent adsorption efficiency and rapid separation from the crude sample matrix by an external magnetic field [3,4]. It is obvious that the excellent absorbent materials must have high specific surface area, chemical stability and a lot of adsorption sites [5]. Atenolol (ATN) is a ß-adrenoceptor antagonist, also known as ß-blocker, which is mainly used for the treatment of heart pathologies (Figure 1). As a drug, it is commonly used to treat angina pectoris, hypertension, cardiac arrhythmia, myocardial infarction (heart attack) and so forth [6-8]. Atenolol is an aminoalcohol and a relatively

polar hydrophilic compound with a pKa of 9.6, water solubility of 26.5 mg mL^{-1} at 37°C, and a log partition coefficient (octanol=water) of 0.23. It is freely soluble in 1M HCl (300 mg mL^{-1} at 25°C) and less soluble in chloroform (3 mg mL^{-1} at 25°C). The ß-blockers are exceptionally toxic and most of them have a narrow therapeutic range, and the difference between their lowest therapeutic and highest tolerable doses is small [7]. Therefore, their determination in biological human fluid-like urine and plasma or pharmaceutical samples is vitally important. Thus far, several methods have been reported for the determination of atenolol, including Nano-liquid chromatography-mass spectrometry [6,7], potentiometeric method [7], differential pulse voltammetric method [8-10], high-performance thin-layer chromatography [11], reverse-phase high-performance liquid chromatography with UV detector [12-15] capillary zone electrophoresis [16] fluorimetry, because of its advantages over the other techniques such as good precision, sensitivity, has been used for most of the drugs [17] spectrofluorimetry can be applied for determining atenolol since it presents natural fluorescence [18-20]. Moreover, fluorescence spectroscopy is intrinsically sensitivity and instruments are easily available [21,22], Additional clean-up is necessary before the chromatographic analysis. However, special SPE materials avoid this problem by providing a selective extraction. Most polymers prepared by non-covalent imprinting have been synthesized using highly functionalized templates with amino, carboxyl, keto, or amino groups. The aim of the present work was to develop a new MSPE technique based on the adsorption properties and selectivity of the SMA regarding the target analytes that could be significantly improved in the presence of 3-aminbenzoic acid (ABA) and 3-aminopropyltriethoxysilane (APTES) and that could demonstrate its applicability for selective magnetic extraction of (ATN) from human serum and pharmaceutical waste-water samples prior to spectrofluorometric determination at λ_{em} = 250 nm after excitation at 230 nm. Comparing the fluorescence spectra of (ATN) before and after MSPE procedure confirmedthe (ATN) inclusion and the effect of the poly (ABA-SMA)-APTES-Fe_3O_4as sorbent on the fluorescence intensity of the analyte. Decrease in the analytical signal of (ATN) in the presence of the sorbent during the MSPE procedure can be related to the higher collision fluorescence quenching of (ATN) due to its high aggregation degree in the sorbent pores. Therefore, in this work, we investigated the first application of modified SMA copolymer magnetite Nano-hybrid as a novel sorbent for MSPE of atenolol.

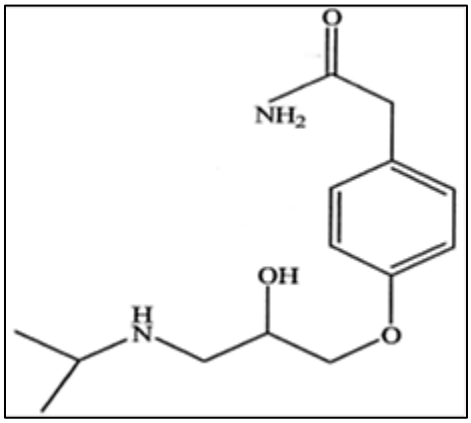


Figure 1: Structure of the b-blocker atenolol

EXPERIMENTAL SECTION

Apparatus and Instruments

Fluorescenc espectra and intensity measurements were carried out using a FP-6500 JASCO Corporation (Tokyo, Japan) spectro-fluorometer with a wavelength range of 220–730 nm (with1nm intervals) for excitation and emission. The instrument equipped with a 150W xenon lamp, 1.0 cm quartz cell, Peltier thermo statted single cell holder (model ETC-272), and supported with PC-based Windowss Spectra Manager TM software for JASCO Corporation version 1.02. The slit widths for both excitation and emission were set at 5 nm and the fluorescence spectra were recorde data scan rate of 250 nm min^{-1}. In order to structural study of the nano-particles, XRD measurements were performed on a Bruker AXS model D8 Advance (Karls- ruhe, Germany) instrument with Cu-Kα radiation source (1.54 Å) between 2 and 701 generated at 40 kV and 35 m. A at room temperature. Samples for XRD were ground in to powder and then pressed flat in the samples lot. In addition, FT-IR spectra (4000-400 cm^{-1}) were recorded on a Bruker model Vector 22 (Ettlingen, Germany) Fourier transformin frared spectrometer using the KBr disk method with a ratio sample/KBr of 1:100 by mass. As scanning electron microscope (SEM), modelL EO1430 vp (CarlZeiss,

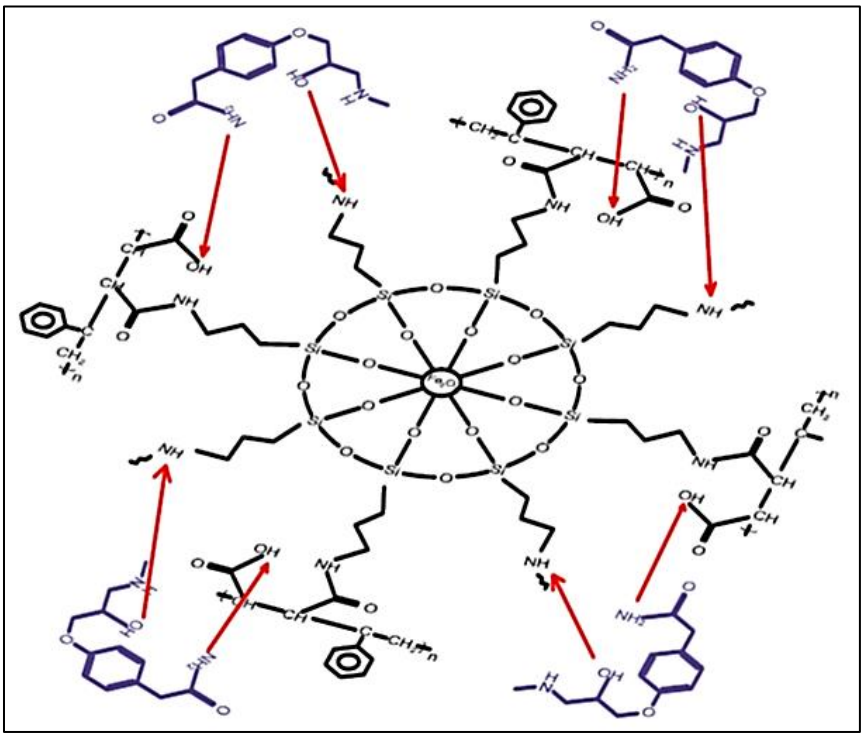
Geramany), was additionally used to examine the morphological characteristics of the sorbent. An ultrasonic bath (SONICA, Italy) was used to disperse the adsorbentin sample solution vials. A shaker (ParsAzmaCo., Iran) was used for controlled stirring the sample solution vial sina dsorption and desorption steps. The pH values were measured with a Metrohm digital pH-meter model 827 (Herisau, Switzerland) supplied with a glass-combined electrode. An electronic analytical balance, Mettler Toledo model PB 303 (Greifensee, Switzerland) was used for weighting the solid materials.

Standard Solutions and Reagents

All chemicals used were of analytical reagent grade and all solutions were prepared with high purity deionized water Artemia research center (Urmia, Iran). $FeCl_3$ $6H_2O$, $FeCl_2$ $4H_2O$, NH_3, H_2O and other chemical reagents was purchased from Merck (Darmstadt, Germany). A stocks tandard solution of 1000 mgL^{-1} atenalol (Sigma- Aldrich, St.Loius, MO, USA) was prepared by dissolving appropriate amounts of atenalol in deionized water. Working standard solutions were obtained daily by suitable stepwise dilution of the stock solutions with deionized water and shaking them just prior to use. All reagents and solvents including ethanol, sulfuric acid, acetic acid, hydrochloric acid, THF, $SOCl_2$, 3- aminopropyltriethoxysilane (APTES), propanediamine, triethylamine (TEA), trifluoroacetic acid (TFA) styrene, maleic anhydride, 3-aminobanzoic acid (ABA) and all the salts used, were purchased from Merck Company. To adjust the pH of the solution, 0.01M acetic acid-acetate buffer (pH 3-6.5) or 0.01M phosphate buffer (pH 6.5-9) were used wherever suitable. The pipettes and vessels used for the trace analysis were kept in 15% (v/v) nitric acid at least overnight and subsequently washed three times with deionized water prior to use.

Synthesis of modified (poly(ABA-SMA)-APTES-Fe_3O_4) copolymer and magnetic nano-composite:

The SMA copolymer was prepared by free radical polymerization of maleic anhydride and styrene at 70°C in the presence of benzoyl peroxide as initiator (Scheme 1) [23].



Scheme 1: Complexation of poly (ABA-SMA)@APTES-Fe3O4 MNC with atenolol

Sample Preparation

Human serum samples:

All experiments on humans were performed in compliance with the relevant laws and institutional guidelines approved by the Medical Committee of the Urmia University of Medical Sciences, Urmia, Iran. Human serum samples were selected as real samples for analysis by the presented method. Toprecipitate and remove interfering proteins, the serum samples were diluted 1:4 with acetonitrile and centrifuged for 10 min at 4,000 rpm [24]. Then, 1

mL of the supernatant was diluted 200 times and subjected to extraction and determination by following the procedure.

Pharmaceutical waste water samples:
Pharmaceutical waste-water samples were collected from Pharmaceutical Manufactory Effluents in Tehran, Iran. These samples were filtered through black bond filter paper and centrifuged to remove any suspended particulate. Then, aliquots of 200 mL from samples were analyzed within 24 h of collection, without other treatments.

Pharmaceutical Preparations
In order to find the average mass of each capsule, five capsules of atenolol were weighed. Then the contents were powdered and mixed. A portion of 10.0 mg of the powder was accurately weighed and dissolved in 10 mL of 0.1 mol L^{-1} sodium hydroxide solution and filtered into a100 mL volumetric flask [25]. The residue was washed several times with deionized water and the flask was then made up to the mark with the water. A suitable aliquot of this solution was diluted stepwise and taken for magnetic solid-phase extractionspectrofluorometric determination of atenolol.

General Procedure
Nano-sorbent of 150 mg was placed in a 250 mL glassware beaker. Then, a 200 mL portion of the standard or sample solution containing atenolol in the range of 0.1-60 μg L^{-1} was transferred into a beaker. To disperse the Nano-sorbent homogenously through the whole solution, the beaker was placed in an ultrasonic bath for 1 min. The adsorption of atenolol on the sorbent was performed under continuous mechanical stirring of the mixture by a shaker for 5 min at room temperature. Finally, the sorbent was gathered at one side of the beaker under an external magnetic field (Nd-Fe-B, 10,000 Gs), and the clear supernatant was directly decanted. To desorb the extracted analyte, 2 mL of HCl 2M+ TEA 1%+ TFA 1% solution was added on the isolated Nano-sorbent. After shaking for 5 min, the Nano-sorbent particles were gathered again with the aid of a magnet. The clear solution of the eluent containing atenolol was transferred into aspectrofluorometric cell and fluorescence intensity of the analyte was measured at λ_{em}=250 nm after excitation at 230 nm.

RESULTS AND DISCUSSION

Characterization of Poly (ABA-SMA)-APTES-Fe_3O_4 Magnetic Nano-Composite
FT-IR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. Figure 2 shows the FT-IR spectra of (a) Fe_3O_4 NPs, (b) APTAS-Fe_3O_4 NPs, (c) poly(ABA-SMA), and (d) poly(ABA-SMA)-APTES-Fe_3O_4 MNC. In Figure 2 the absorption bands at around 672 cm^{-1} can be ascribed to the Fe−O stretching vibration of Fe_3O_4. In addition, O-H stretching vibration around 3422 cm^{-1} and O-H deformed vibration at 1627 cm^{-1} are observed in these spectra, suggesting that −OH groups coat on the surface of Fe_3O_4 NPs as reported [26]. The presence of APTMS on the surface of Fe_3O_4 NPs is also certified by the bands at 1120 and 1027 cm^{-1} which are due to Si−O stretching vibrations (Figure 2). This reveals that the covalent bonds of Fe−O−Si are formed after modification of Fe_3O_4 NPs through silanization reaction with APTMS. The broad band at 3421 cm^{-1} is referred to the N−H stretching vibration which can be overlapped by the O−H stretching vibration band. Moreover, the characteristic peaks of C−H stretching vibrations at 2855 and 2923 cm^{-1} confirm the presence of an anchored propyl group. Comparison of the FT-IR spectra of poly (ABA-SMA) (Figure 2) and poly(ABA-SMA)-APTES-Fe_3O_4 MNC. Figure 2 indicates that characteristic absorption bands of the anhydride linkage of poly(ABA-SMA) at 1735, 1784, and 1856 cm^{-1} disappear after reaction with the APTMS-Fe_3O_4 NPs. As shown in Figure 2, in the case of poly (ABA-SMA)-APTES-Fe_3O_4 MNC, the absorption band at 1728 cm^{-1} is related to the amide groups and the peaks at 1635 and 3419 cm^{-1} confirm the presence of the carboxylic groups.
The powder X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. To study of the crystal structure of poly (ABA-SMA)-APTES-Fe_3O_4 MNC, the XRD patterns of the as-prepared poly (ABA-SMA), Fe_3O_4 NPs and poly(ABA-SMA)-APTES-Fe_3O_4 MNC are shown in Figure 3, respectively. As shown in Figure 3, there is only a broad diffraction peak at about $2\theta = 20^{\circ}$, which is assigned to the (0 0 5) reflection of poly (ABA-SMA), indicating the amorphous nature of the copolymer. The XRD pattern of Fe_3O_4 NPs illustrates their cubic spinel structures, and the existence of sharp and intense peaks proves the formation of highly crystalline Fe_3O_4 NPs (Figure 3).
The XRD pattern of the synthesized poly (ABA-SMA)-APTES-Fe_3O_4 MNC shows diffraction peaks at the Bragg angles of about 30.21, 35.51, 43.21, 53.61, 57.11 and 62.91, which are respectively attributed to the (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) facets of the cubic spinel crystal planes of Fe_3O_4 (JCPDS No.19-0629) (Figure 3).

So, the existence of Fe_3O_4 NPs in poly (ABA-SMA)-APTES-Fe_3O_4 is confirmed, while the (0 0 5) reflection peak of poly(ABA-SMA) is almost vanished. It may be due to the fact that after covering with Fe_3O_4 NPs, the poly (ABA-SMA) particles cannot pile with each other anymore to form crystalline structures [27,28].

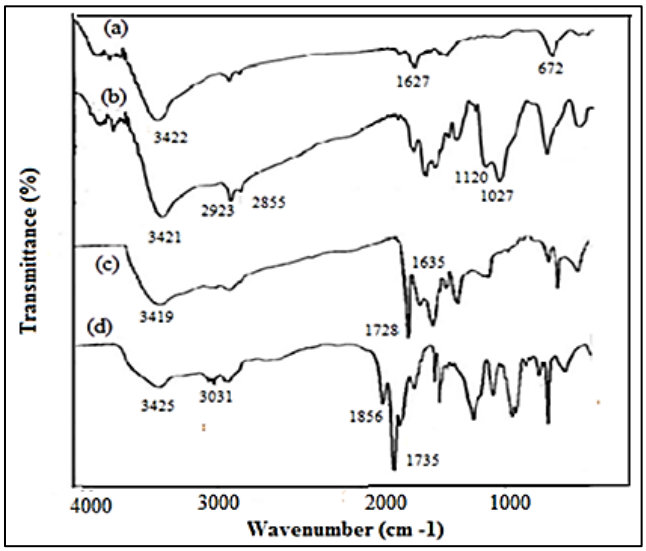


Figure 2: FT-IR spectrum, a) Fe_3o_4 b) APTES (3-aminopropyltriethoxysilane), c) APTES/SMA, d) SMA

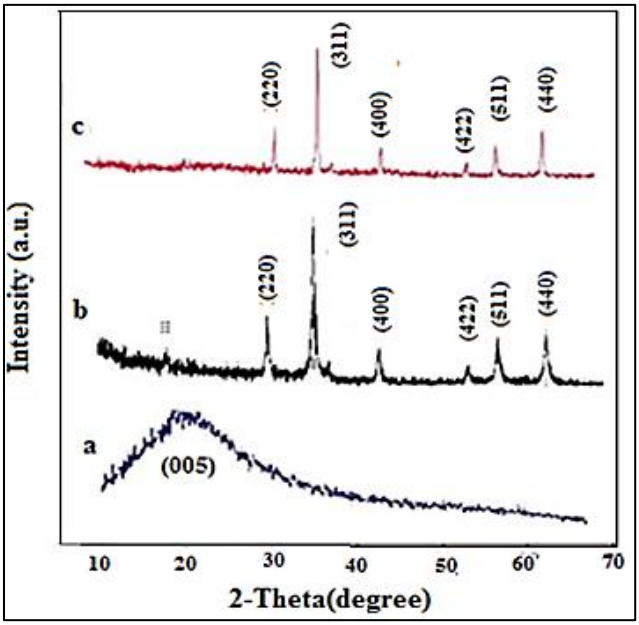


Figure 3: XRD pattern of (a) SMA (b) poly (ABA-SMA)@APTES-Fe_3O_4 MNC (c) Fe_3O_4 MNC nanohybride, respectively

Additionally, FESEM was used to prospect the morphology of the synthesized particles. The morphology of poly (ABA-SMA) appeared that the co-polymer has tumultuous nature and the average diameter of the observed particles in FESEM image could be estimated under 100 nm with reasonable uniformity and grainy shape. FESEM image of poly(ABA-SMA)-APTES-Fe_3O_4 demonstrated an condensed that consists of MNC crystallites which were collected as small pseudo-spherical particles with approximate sizes in the range of 10-50 nm were stacking with each other, which makes plate-like morphology (Figure 4).

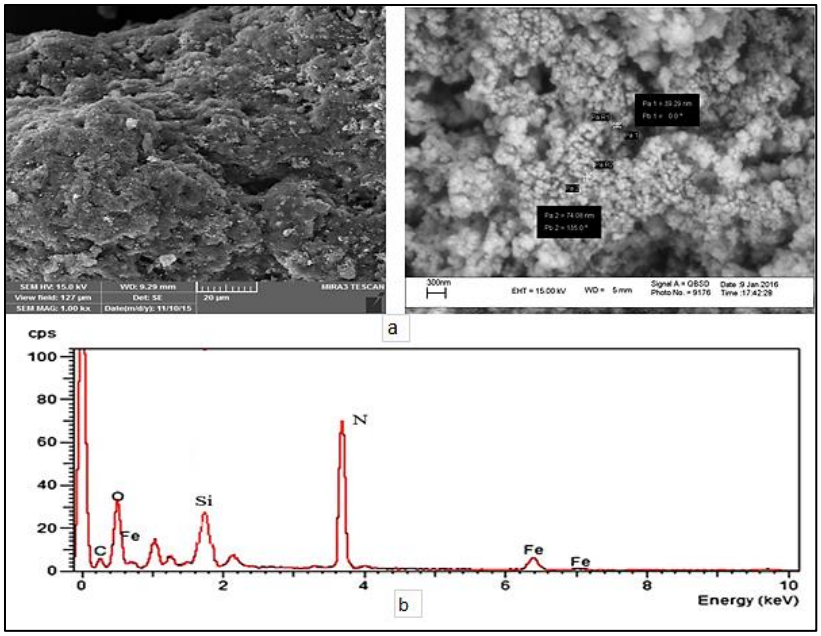


Figure 4: a) FESEM image of poly (ABA-SMA)-APTES-Fe_3O_4 MNC b) EDX spectrum of Modified (SMA)-APTES-Fe_3O_4 nanoparticles

Fluorescence Spectra

The fluorescence spectrum of the blank and atenolol in the presence and absence of the sorbent are shown in Figure 5, respectively. It was shown that the emission spectrum of (ATN) was resolved into a peak at 300 nm (Figure 5). The fluorescence intensity of (ATN) was decreased when (SMA)-APTES-Fe_3O_4MNC was added to the system. In this case, the maximum emission wavelength was 300 nm (Figure 5). Moreover, (ATN) incorporated into the (SMA)-APTES-Fe_3O_4MNC exhibits low fluorescence intensity, which is about five times lower than that of (ATN) solution without adding Nano-sorbent. The comparison of the emission spectrum of (ATN) solution with the solution incorporated into (SMA)-APTES-Fe_3O_4MNC confirmed the sensitizing effect of the (SMA)-APTES-Fe_3O_4MNC on the fluorescence intensity of (ATN). The lowering of fluorescence intensity of (ATN) in the presence of the (SMA)-APTES-Fe_3O_4MNC matrix can be attributed to a successful MSPE of (ATN) from aqueous solution, leading to increase in probable quenching processes that usually occur in the aqueous solutions due to high degree of (ATN) aggregation in the (SMA)-APTES-Fe_3O_4 porosity.

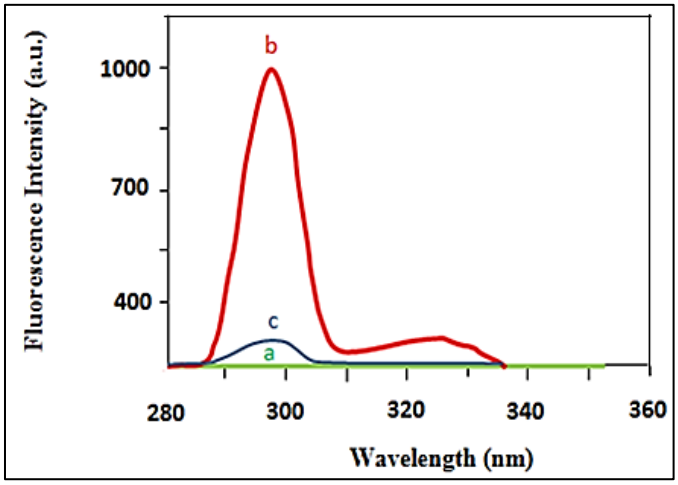


Figure 5: Fluorescence spectrum of the (a) blank, (b) atenolol in the presence (c) atenolo in the absence of the sorbent

Optimization of Magnetic Solid Phase Extraction Conditions

To evaluate the capability of the presented MSPE method for separation and pre-concentration of atenolol, several variables affecting the extraction efficiency including pH, the amount of magnetic Nano-sorbent, the sample volume, and the type of eluent on the extraction efficiency, were studied and optimized. Each parameter was varied

individually to optimize the experimental conditions. A 10 µg L^{-1} solution of atenolol was used for all measurements and each experiment was performed three times.

Effect of pH

Due to the structure of the drug and its size and functional groups such as NH-and OH, the drug can be physically in the polymeric 3-dimensional space, sealed and placed inside the polymer. In addition, according to the conditions of the pH several chemical bonds can be established between the drug and the functional groups of the polymer that are in the pH conditions as follows: The influence of the pH value on the separation/pre-concentration of atenolol was studied by adjusting the pH values of sample solution in the range of pH 5-12 using either diluted HCL or NaOH solution or both. Solutions with pH<4 were not tested because the Fe_3O_4 Nano-particles dissolves in acidic pH. According to the obtained results, the recovery increases with increasing the pH from 4 to 7 and remains constant between pH 7 and 9 before decreasing at pH values higher than 10. By increasing the pH, coordination ability of amine and hydroxile groups in the Nano-sorbent structure may increase, leading to high absorption and desorption of the analyte and, at pH>10, There is no change in absorbance in alkanes higher than 10 (strong alkali) with increasing hydroxyl groups. Accordingly, pH 7 was selected as the optimum pH for further experiments, and phosphate buffer solution (0.1 mol L^{-1}) was used for adjusting the pH (Figure 6).

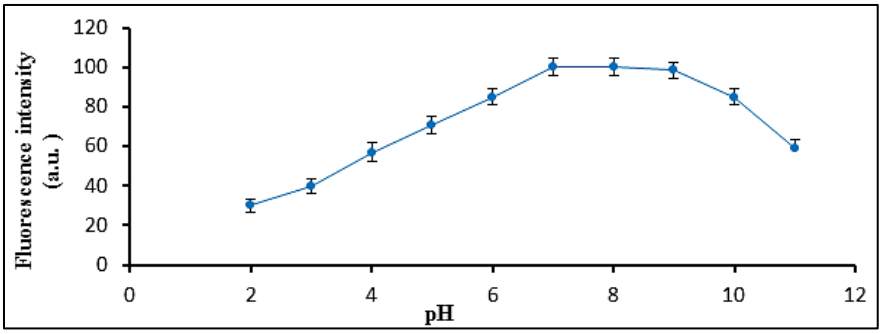


Figure 6: Effect of pH on the extraction efficiency of atenolol utilized conditions: atenolol 10 µg L^{-1}; poly(ABA-SMA)-APTES-Fe3O4MNC (magnetic nano sorbent), 200 mg; 3 mL of (HCl 2M+ TEA 1%+ TFA 1%) as eluent; centrifugation time, 20 min

Effect of Amount of Nano-hybrid

The effect of the amount of **(**SMA)-APTES-Fe_3O_4 Nano-hybrid on the sorption of atenolol was examined in the range of 50-300 mg. The results demonstrated that quantitative recoveries (>95%) of the working analyte were observed when the synthesized Nano-hybrid was used above 100 mg. Therefore, in the presented procedure, 150 mg of (SMA)-APTES-$Fe_3O_4$was recommended (Figure 7).

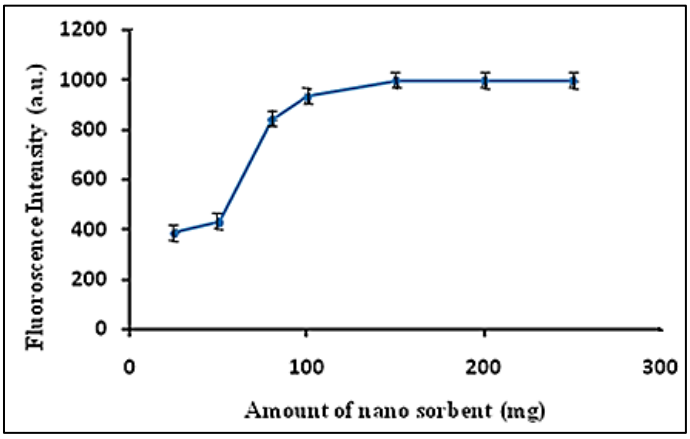


Figure 7: Effect amount of nano sorbent on the extraction efficiency of atenolol utilized conditions: atenolol 10 µg L^{-1}; (pH 7.0); 3 mL of (HCl 2M+ TEA 1%+ TFA 1%) as eluent; centrifugation time, 20 min

Optimization of Elution Conditions

The nature of the eluent is of prime importance and should optimally meet three criteria: efficiency; selectivity; and compatibility. In addition, it may be desirable to recover the analytes in a small volume of solvent to ensure a significant enrichment factor. In this work, elution of the retained atenolol from (SMA)-APTES-Fe_3O_4 surface was examined using various reagent solutions and the results are shown in Figure 5. As can be seen, the best recovery was achieved when HCl 2 M+ TEA 1%+ TFA 1% was used as an eluent. The effect of elution volume (1.0-4.0 mL) on the recovery was also investigated. The recovery of the analyte increased by increasing the volume of the eluent up to 2 mL and remained constant thereafter. So, to achieve the highest pre-concentration factor, 2 mL of the eluent was chosen as the optimum value (Figure 8).

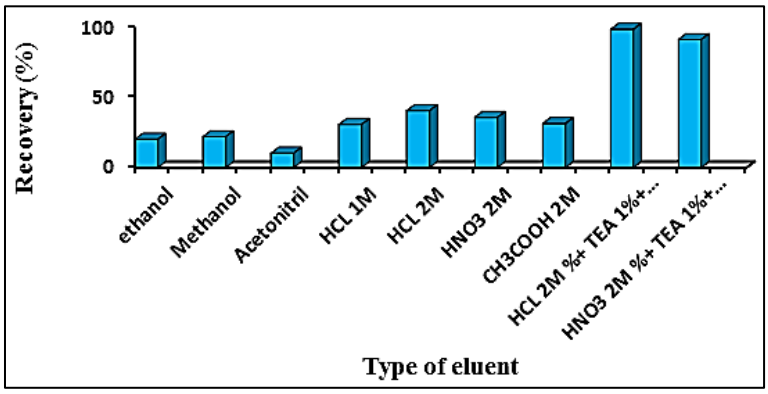


Figure 8: Effect of eluent type and concentration on the recovery of 10 µg L^{-1} atenolol. Experimental conditions: pH; 7, amount of sorbent; 150 mg, sample volume; 50 mL)

Effect of Sample Volume

The possibility of enriching low concentrations of atenolol from large volumes of samples was examined by studying the effect of sample volume on the recovery of the analyte. For this aim, the volumes of sample solutions containing 10 µg L^{-1} atenolol were diluted to 25-400 mL and the analyte was pre-concentrated on (SMA)-APTES-Fe_3O_4 Nano-hybrid by applying the presented MSPE procedure. As shown in Figure 9, a quantitative recovery of atenolol was obtained up to 200 mL of sample solution; above 200 mL, the recovery decreased. So, by analyzing 2 mL of the final solution obtained after the pre-concentration of 200 mL of sample solution, a pre-concentration factor of 100 was obtained.

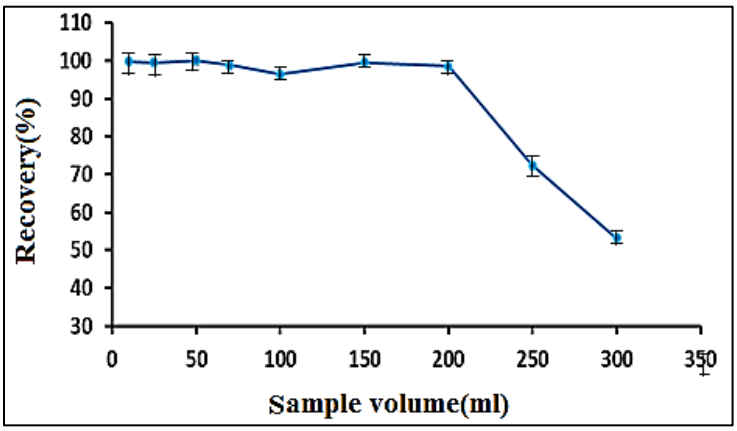


Figure 9: Evaluation of the extraction efficiency of atenolol by using the different sample volumes and constant amount of poly (ABA-SMA)-APTES-Fe_3O_4MNC as sorbent, utilized conditions: atenolol, 10µg L^{-1}; phosphate buffer (pH 7.0); MNC, 150 mg; eluent volume 2 mL of (HCl 2M+ TEA 1%+ TFA 1%); centrifugation time, 5 min

Effect of Adsorption/Desorption Time

Due to the superparamagnetic property of the Nano-sorbent, the sorbent could be rapidly separated from the sample solution in about 2 min using an external magnetic field instead of filtration or centrifugation. However, to achieve satisfactory recovery and short analysis time during the separation and determination of the analyte, the effect of adsorption/desorption time on the recovery of atenolol was investigated. Both the adsorption and desorption time

were varied in the range of 1-20 min. It was observed that 5 min was sufficient for each step to achieve satisfactory recoveries during determination of atenolol.

Regeneration, Reusability and Adsorption Capacity of Nano-sorbent

The potential regeneration and stability of the sorbent was also investigated. The Nano-hybrid could be reused after regenerating with 2 mL of HCl 2M+ TEA 1%+ TFA 1% and 50 mL deionized water. Moreover, based on the obtained results, the prepared (SMA)-APTES-Fe_3O_4 Nano-hybrid can be reused more than 50 times without obvious loss of analytical performance and magnetic properties. The adsorption capacity (q_e, mg g^{-1}) of the Nano-sorbent for atenolol was calculated using the following equation:

$$q_e = V\ C_0 - C_e / W \quad (1)$$

Where C_0 (mg L^{-1}) and C_e (mg L^{-1}) are the initial and equilibrium concentrations of the analyte in aqueous solution, respectively, V (L) is the volume of sample solution, and W (g) is the mass of used (SMA)-APTES-Fe_3O_4Nano-hybrid. For this aim, 300 mg of the Nano-sorbent was added to 100 mL of solution containing 15 mg L^{-1} of the analyte and sonicated for 1min to obtain fully-dispersed Nano-hybrid particles throughout the sample solution. The extraction process was continued for 45 min by stirring the solution with a mechanical stirrer.Then, the magnetic Nano-hybrid was isolated from the solution by a magnet. Finally, the concentration of the analyte in the supernatant was determined by spectrofluorometric approach. As a result, the adsorption capacity of the Nano-sorbent for atenolol was found to be 96.7 mg g^{-1}, according to Equation 1.

Optimized experimental parameters and analytical characteristics of the method are given in Table 1. Under these experimental conditions, the analytical features of the presented method such as the linear range of the calibration graph, the limit of detection (LOD) and limit of quantification (LOQ), and the accuracy and the precision, were examined. The calibration graph was linear in the range between 0.1 and 60 μg L^{-1}, with a correlation coefficient of 0.9989. The regression equation was I_F=135.021C + 32.00, where I_F is the fluorescence intensity and C is the concentration of atenolol in the sample solution in μg L^{-1}. The LOD and LOQ, defined as 3 S_b/m, and10 S_b/m (where S_b is the standard deviation of the blank and m is the slope of the calibration curve) were 0.32 μg L^{-1} and 0.18 μg L^{-1}, respectively. The precision of the method was evaluated by repeated analysis of atenolol during the course of experimentation on the same day and on different days under the optimized experimental conditions. For both intra-day and inter-day variation, solutions of atenolol at concentrations of 10 μg L^{-1} were determined intriplicate. The % RSD for analysis was found to be 1.09%. In order to explore the reliability of the presented method, we used the magnetic nano sorbent to extract (ATN) from different samples of pharmaceutical waste water, human serum and atenalol capsules with a nominal content of 500 mg.

The results are given in Table 2. Due to unavailability of certified reference materials for atenolol to test the validity of the method, recovery experiments were carried out by spiking the samples with different amounts of HCl 2M+ TEA 1%+ TFA 1% solvent.

Method Validation and Analysis of Real Samples

To explore the reliability of the presented method, the method was successfully applied to determine atenolol in various samples including pharmaceutical wastewaters and human serum samples along with one pharmaceutical product (atenolol capsules with a nominal content of 500 mg). The results are given in Table 2. Due to unavailability of certified reference materials for atenolol to test the validity of the method, recovery experiments were carried out by spiking the samples with different amounts of atenolol before sample preparation determination by spectrofluorometry. As can be seen in Table 2, relative recoveries between 96.0% and 103.0% were obtained, which confirm the accuracy of the presented method.

Table 1: Optimized experimental parameters and analytical characteristics of the presented method for for atenolol determination

Parametr	Unit	Value
Experimental conditions	-	-
Amount of sorbent	mg	150
Sample volume	ml	200
Eluent volume	ml	2
Determination conditions	-	
Excitation wavelength	nm	230
Emission wavelength	nm	250
Analytical parameters	-	-
Linear range	μg L^{-1}	0.1-60
Intercept		32
Slope		135.021
Correlation coefficient	-	0.998
Enrichment factor	-	100
Limite of detection	μg L^{-1}	0.32
72% Relative standard deviation (RSD)	%	1.09

[a] Calculated as three times the standard deviation of the blank signal divided by the slope of the calibration graph; [b] Value in parentheses is the atenolol concentration (μg L^{-1}) for which the RSD was obtained; [c] Calculated as the ratio between the volume of the initial aqueous solution and the final elution volume

Table 2: Determination of atenolol in different samples

Samples	Added atenolol (μg L^{-1})	Found atenolol (μg L^{-1})	Recovery(%)
Wastwater samples[a]	-	-	-
Sample 1	-	0.42 ± 0.05	-
	0.5	0.93 ± 0.09	101.1
	2	2.38 ± 0.09	98.34
Sample 2	-	1.82 ± 0.08	-
	0.5	2.4 ± 0.06	103.44
	2	3.79 ± 1.0	99.21
Sample 3	-	-	-
	0.5	0.48 ± 0.05	96
	2	2.05 ± 0.08	102.5
Serum samples [b]			
Sample 1	-	-	-
	0.5	0.49 ± 0.87	98
	2	2.04 ± 1.03	102
Sample 2	-	-	-
	0.5	0.51 ± 1.0	102
	2	2.06 ± 1.1	103
	Nominated amount (mg per capsule)	Found amount (mg per capsule)	Recovery (%)
Atenolol capsule[c]	500	398.3 ± 0.4	99.5

[a] Collocated from Pharmaceutical Manufactory effluents, Tehran, Iran; [b] Obtained from Dr Ansari Lab, urmia, Iran; [c] Obtained from Dr.ABIDI Pharmaceutical Co., Tehran, Iran

Study of Interferences

In view of the selectivity provided by spectrofluorometry, the possible interferences can mostly be attributed to the MSPE step. In order to demonstrate the selectivity of the developed extraction system for the determination of (ATN), the effect of different types of concomitant including pH of the sample solution, amount of the sorbent, extraction and desorption times, sample volume, and elution conditions on the recovery of (ATN) was also investigated. The tolerance limits of the interferences, definedas the maximum concentration of the foreign compound causinga change in the analytical signal no higher than ± 5%, are given in Table 3. It can be seen that most of the examined cations and anions and other drugs did not interfere with the extraction and determination. These results permit the application of (SMA)-APTES-Fe_3O_4as an efficient and selective dispersive solid-phase extractor for the interference-free extraction and determination of (ATN) in different biological samples before sample preparation and determination by spectrofluorometry. As can be seen in Table 3, relative recoveries between 96.0% and 104.0% were obtained, which confirm the accuracy of the presented method.

Table 3: Tolerance limits of potentially interfering ions in the determination of atenolol (10 μ g l^{-1})

Interfering ions	Interferent to analyte ratio
Na^{+}, Cs^{+}, Mg^{+2}, NO_3^{-}, CO_3^{-}, Sr^{+2}, Sn^{+2}, PO_4^{-2}, Cl^{-}, Br^{-} Fe^{+3}, Cl^{-}, SO_4^{-2}, CH_3COO^{-}, CO_3 $^{-2}$, PO_4 $^{-3}$	3000:01:00
Sodium chloride, aspartame, methadone, glucose, tryptophan, glycine	13-04-1900 04:01
Dopamine, Meroxan, carbamazepine	1500:01:00
Cephalexin, Naproxen, Nortriptilina, Cephixim	1000:01:00

Comparison of the Presented Method with other Pre-Concentration Procedures

Table 4 compares analytical data from this method with other techniques reported previously regarding the pre-concentration and determination of atenolol. As can be seen, the limit of detection and other parameters of the presented method are better than those of the other procedures in Table 4. This method has some advantages, such as a lower detection limit, a higher pre-concentration factor, simplicity and low cost, and it is environmentally friendly. Accordingly, the presented method can be introduced as ultra-trace analysis of other pharmaceutical compounds in biological and environmental samples.

Table 4: Comparison of the proposed method with other polymers as sorbent with similar methods

Technique Extractant	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	RSD(%)	Sample volume (ml)	Ref
Voltammetric (μmol L^{-1})	4-100	3.16	-	-	[8]
CE[1]	50-400	27	2.5	-	[16]
HPLC/ Fluorescence[2]	10 -1,000		6.4	-	[28]
HPLC	25-800	10	-	-	[29]
LVI-LC–LC-FD[3]	0.2-20	0.002	0.8-9	2	[30]
HPLC	5-150	1.5	1.92	0.5	[31]
Electrochemical	0.4-80 μM	26.6	1.38	-	[32]
HPLC	50-750	5	-	0.5	[33]
Fluorescence	0.1-60	0.32	1.09	100	This work

[1]Capillary electrophoresis; [2]High-performance liquid chromatography-Fluorescence detection; [3]Coupled-column liquid chromatography separation procedure (LC–LC), large volume injection (LVI) and fluorescence detection (FD)

CONCLUSION

In this research, layered double hydroxides have been introduced as a class of inorganic Nano-sorbents in dispersive solid phase micro-extraction methodology. The experimental results indicate that trace levels of atenolol could be extracted from aqueous solutions and directly determined by spectrofluorometry. The (SMA)-APTES-Fe_3O_4Nano-hybrid in the form of solid phase was successfully used for selective extraction of atenolol from human blood serum samples. The presented method was proved to be simple, rapid, with good extraction efficiency and environmentally friendly. No additional cleanup steps are required, thus MSPE saves time, labor, money and solvent use compared with the tedious traditional SPE. In addition, it was shown that combination of novel MSPE procedure with spectrofluorometric detection represented excellent selectivity, repeatability, simplicity that could be used in purification and in situ determination of (ATN) in many intricate matrices.

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