# Solid Phase Extraction Using Modified Magnetic Iron Oxide Nanoparticles for Extraction and Spectrofluorimetric Determination of Carvedilol in Human Plasma Samples

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**ABSTRACT:** *A new analytical approach was developed involving magnetic solid–phase extraction and spectrofluorimetric determination of carvedilol in human plasma samples. A plasma sample was prepared and adjusted to pH 8.2–10, then carvedilol was quickly extracted using iron oxide magnetic nanoparticles modified by the surfactant cetyltrimethylammonium bromide and determined applying spectrofluorimetry at 354 ± 3 nm after excitation at 241 ± 3 nm. Experimental conditions, such as the amount of nanoparticles and cetyltrimethylammonium bromide, pH value, standing time and desorption solvent type and volume have been adjusted to optimize the extraction process and to obtain analytical characteristics of the method. Linearity was observed in the analyte concentration range of 2.0–125 ng/mL with correlation coefficients (r) of 0.999. The method showed good precision and accuracy, with intra– and inter–assay precisions of less than 7.0% at all concentrations. Standard addition recovery tests were carried out, and the recoveries ranged from 94.4% to 100.7%. The limits of detection and quantification were found to be 0.67 and 2.24 ng/mL. The method was applied to the determination of carvedilol in human plasma samples.*

**KEYWORDS:** *Carvedilol; Magnetic iron oxide nanoparticles; Plasma; Solid phase extraction; Spectrofluorimetry.*

## INTRODUCTION

Carvedilol, 1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy) ethylamino]propan-2-ol, is a nonselective adrenergic blocking agent with 1-blocking activity indicated for the treatment of hypertension and mild or moderate heart failure of ischemic or cardiomyopathic origin [1]. Carvedilol is administered as a racemate, but the enantiomers exhibit different pharmacological effects: both enantiomers exhibit similar

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$\alpha_1$-blocking activity, but only the S(−) enantiomer possesses β-blocking activity [2]. Carvedilol is highly bound to protein in plasma (98%). In urine, 2–4% of the dosage is excreted unchanged [3].

Several extraction methods such as Solid Phase Extraction (SPE) and Solid Phase MicroExtraction (SPME) [4-8], Liquid-Liquid Extraction (LLE) [9-13], Dispersive Liquid-Liquid MicroExtraction (DLLME) [14-17], Liquid Phase MicroExtraction (LPME) based on ionic liquids [18,19], Stir Bar Sorptive Extraction (SBSE) [20] and Vortex-Assisted Liquid-Liquid Extraction (VA-LLE) or Vortex-Assisted Liquid-Liquid MicroExtraction (VA-LLME) [3,21] have been used for the extraction of carvedilol from biological samples. Its determination has been done by different techniques including High Performance Liquid Chromatography (HPLC) [3-7, 9, 15, 16, 20], HPLC-Mass Spectrometry (HPLC-MS) [2,11,12], Capillary Electrophoresis (CE) [13,14], spectrofluorimetry [17-19] and micellar electrokinetic chromatography [21]. Several other methods including spectrophotometry [12, 22], chemiluminescence [1] and potentiometry [23] have been reported for the determination of carvedilol in biological fluids.

LLE and SPE methods used for the extraction of carvedilol, suffered from many drawbacks such as consumption of large amounts of organic solvents or use of lengthy extraction procedures. Potential variability of SPE packing, irreversible adsorption of some analytes on SPE cartridges, and more complex method development are some of the drawbacks of SPE method [15]. Although, SBSE and DLLME generally provided better enrichments compared to the traditional extraction methods, however they have some drawbacks. For example the low partition of the analytes into the extraction solvent by using of dispersive solvents and the use of high density and toxic organic solvents are the disadvantages of DLLME [3]. A problem associated with SBSE is the limitation of the SBSE coatings available. Another limitation of SBSE method is the presence of memory effect during desorption step using organic solvent [20].

Recently, novel sample preparation techniques based on Magnetic NanoParticles (MNPs) have attracted extensive attention. A distinctive superiority of this technique is that magnetic materials can be readily isolated from sample solutions by the application of an external magnetic field. Moreover, the high dispersibility and easiness of surface modification also make them widely applicable in many other fields [24]. More recently, Magnetic Iron Oxide NanoParticles (MIONPs) were used in sample preparation techniques. Their high surface area and super paramagnetic property provide higher extraction capacity, rapid extraction and ease of separation from large volume samples by employing a strong external magnetic field [25]. Currently, some ionic surfactants have been used for modifying surfaces of MNPs as SPE sorbents. In these SPE methods, the used adsorbents were produced by the adsorption of ionic surfactants such as CetylTrimethylAmmonium Bromide (CTAB) or Sodium Dodecyl Sulfate (SDS) on the surface of magnetic and/or modified MNPs with silica and alumina [26-38]. Covering the surfaces of MNPs with surface active agents can improve the adsorption capacity and efficiency of the extraction. The hydrophobic layers on the surface of MNPs provide suitable conditions for nonpolar components to interact with MNPs [35]. This Magnetic SPE (MSPE) method based on mixed-hemimicelles assemblies has been used for the extraction and preconcentration of a variety of organic [25-36] and inorganic [37-40] compounds from various matrices.

In the present work, CTAB coated MNPs were synthesized and employed as sorbent in NP based SPE of carvedilol from human plasma and its determination by spectrofluorimetry. Fluorescence spectrometry has been used because of its great sensitivity and selectivity as well as its relative low cost. Various factors that influence extraction performance including extraction and elution conditions were investigated and optimized.

## EXPERIMENTAL SECTION

### *Apparatus*

Spectrofluorimetric measurements were done using a Shimadzu RF–5301 PC spectrofluorophotometer equipped with a 150 W Xenon lamp and using 1.00 cm quartz cells. Instrument excitation and emission slits both were adjusted to 3 nm. A centrifuge from Hettich (EBA 20 model/ Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) with 15 mL calibrated centrifuge tubes (Hirschmann, EM techcolor, Germany) was used to accelerate the phase separation process. The pH–meter model M120 (Halstead, Essex, England CO9 2DX) supplied with a glass combined electrode was used for the pH measurements. The mixtures were shacked using a Unimax 1010 Shaker–Inkubator (Heidolph, Germany).

### *Materials*

Chemicals including iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), SDS, ethanol (EtOH), methanol (MeOH), sodium chloride (NaCl), trichloroacetic acid, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were obtained from E. Merck (Darmstadt, Germany). The ionic surfactant CTAB was obtained from Sigma-Aldrich (St. Louis, MO).

A stock solution of 500 µg/mL of carvedilol (gifted by Jalinous Pharm. Co., Tehran, Iran) was prepared by dissolving appropriate amount of pure drug in MeOH and was kept away from the light in a refrigerator at approximately 4°C. Working standard solutions were obtained by appropriate dilution of this stock standard solution. A solution of 2000 µg/mL of CTAB was prepared by dissolving appropriate amounts of this compound in deionized water and diluted up to the mark. All other reagents were of analytical reagent grade or higher. Ultrapure water (Milli–Q Advantage A 10 system, Millipore) was used throughout the work.

### *Preparation and characterization of MNPs*

MIONPs were prepared by the co–precipitation method [41] with some modifications and according to our previous works [36,37]. For this purpose, 4.6 g of $FeCl_3{\cdot}6H_2O$, 2.0 g of $FeCl_2{\cdot}4H_2O$, and 0.85 mL of HCl (12 mol/L) were dissolved in 25 mL of deionized water which was degassed with $N_2$ before use. The resulted clear yellowish green solution was added drop–wise into 250 mL of 1.5 mol/L NaOH solution (heated to 80 °C), under vigorous stirring with $N_2$ passing continuously through the solution during the reaction. Upon addition, the solution turned black and was then stirred magnetically at 1000 rpm for 30 min. After the reaction, the obtained precipitate was separated from the reaction medium by magnetic field, washed with 200 mL of deionized water four times, and then re–suspended in 250 mL of deionized water. The concentration of MNPs in this solution was found to be 1% w/v. As can be seen in Fig. 1, the average particle size of obtained MNPs was less than 42 nm by using a scanning electron microscopy (SEM).

For proving the adsorption of the surfactant to MIONPs the FTIR spectra were plotted, so Fig. 2 displays the IR spectrum for MIONPs alone and CTAB-coated MIONPs. Three characteristic bands can be

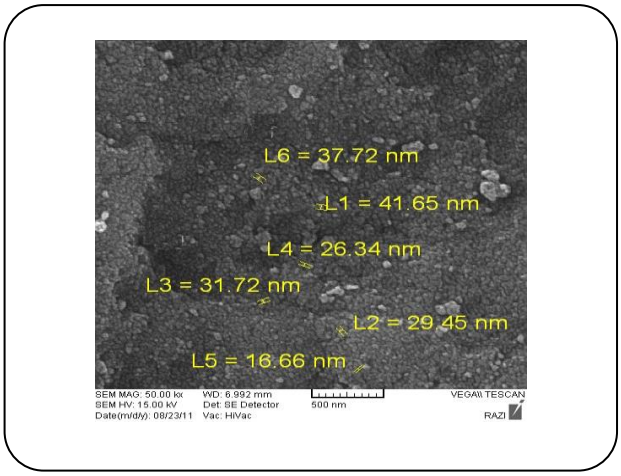


***Fig. 1: The SEM image of dispersed MNPs.***

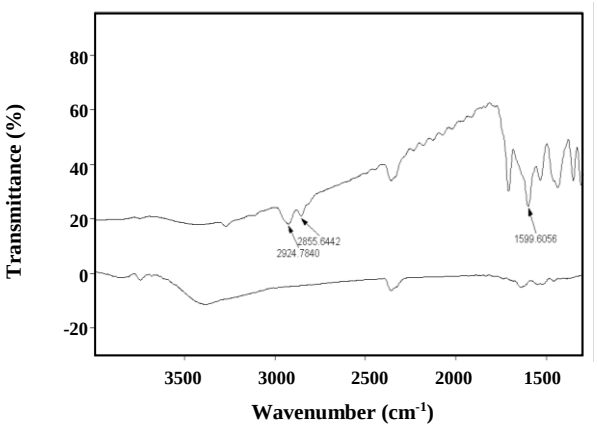


***Fig. 2: IR spectrum of the $Fe_3O_4$ NPs (A) and the CTAB coated $Fe_3O_4$ NPs (B).***

distinguished in the IR spectrum of CTAB coated MIONPs, while not observed in the MIONPs IR spectrum. Compared with the standard spectrum, the adsorption bands at 2924.78 and 2855.64 $cm^{-1}$ could be attributed to the stretching vibration of C–H band, as well as the adsorption band at 1599.60 $cm^{-1}$ could be attributed to the stretching vibration of N-H band, which showed that the surface of MIONPs was successfully modified with CTAB.

### *Procedure for plasma samples*

Drug–free plasma samples were obtained from the Blood Transfusion Organization in Tabriz, Iran, and stored at -20 °C until they were analyzed. This sample named as Quality Control (QC) samples. The samples were treated based on reported methods with some

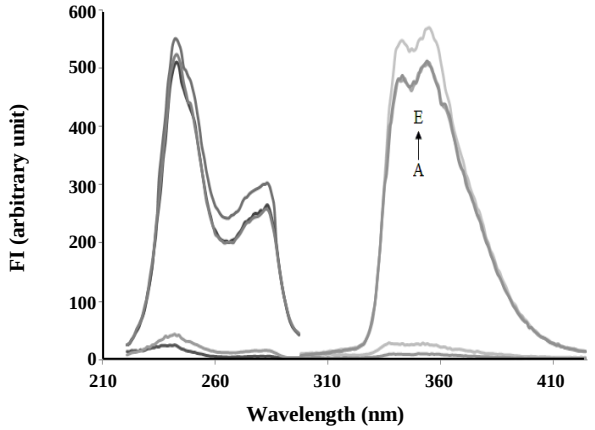


***Fig. 3: Excitation and emission spectra after MSPE: (A) reagent's blank, (B) plasma blank, (C) spiked carvedilol to pooled plasma at 50 ng/mL concentration level, (D) standard solution of carvedilol (50 ng/mL), (E) collected plasma sample (after administration of carvedilol to one volunteer). Conditions: pH 8.2–10; NP (0.14%), CTAB (200 ng/mL), desorption with 2 mL of MeOH, other conditions have been mentioned in the text.***

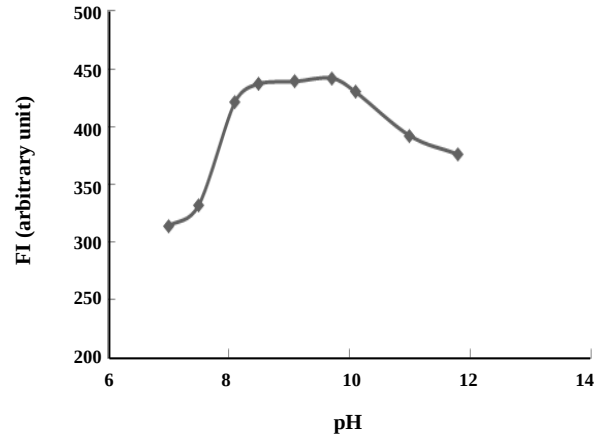


***Fig. 4: The effect of pH on the analytical signal for 50 ng/mL carvedilol. Conditions: NP (0.14%), CTAB (200 ng/mL), desorption with 2 mL of MeOH, other conditions have been mentioned in the text.***

modification [42]. Briefly, ten ml blood sample was taken from a man volunteer (23 years old) in complete blood cell count (CBC) tubes and 2 h after administration of a single oral dose of carvedilol tablet (6.25 mg). After centrifugation at 4000 rpm for 15 min, the plasma was removed and frozen at –20°C before use. A frozen plasma sample was thawed at room temperature, then an aliquot of 3 mL transferred into a 15 mL centrifuge tube and spiked with carvedilol at proper concentration range. For precipitation of plasma proteins, a volume of 3 mL of trichloroacetic acid (1 mol/L) was added. The contents were mixed and centrifuged at 4000 rpm for 15 min. An aliquot of clear supernatant solution in the range 0.5 to 3.0 mL was subjected to MSPE as described in below section.

### *Procedure for MSPE*

An aliquot of 3.0 mL of prepared plasma was placed in a 20 mL beaker and spiked with carvedilol to give a concentration in the range 2.0–125.0 ng/mL. Then, the sample was mixed with 1.4 mL of 1.0% w/v NPs solution and 1.0 mL of CTAB solution. The solution was diluted to approximately 9 mL and pH adjusted between 8.2-10 by adding 1.0 mol/L NaOH solution, then the volume was made up to 10 mL with ultra–pure water. The flask was shaked at 200 rpm for 15 min to complete the extraction process. Then, the CTAB–coated MIONPs were isolated from the solution by using a strong magnet which was placed at the bottom of the beaker. After about 5 min, the supernatant solution was decanted, then the drug eluted from the MIONPs by using 2 mL of MeOH with the aid of stirring at 200 rpm for 5 min. Finally, the fluorescence intensity of the target analyte was measured at 354 ± 3 nm with the excitation wavelength set at 241 ± 3 nm.

## RESULTS AND DISCUSSION

For the MSPE of carvedilol from plasma samples, the influences of different factors on the extraction and elution steps must be studied and optimized. Fig. 3 shows the excitation and emission spectra for carvedilol extracted from aqueous or plasma samples using optimized MSPE conditions that were established in this work.

### *Effect of pH*

In the mixed hemimicelles based SPE, the pH of the sample solution plays a critical role in the hemimicelles formation and the target compound extraction, due to the different charge density of the MIONPs surface and ionic states of analytes at the different working pHs, respectively [24,25,36,37]. In this study, the effect of pH was examined by varying pH between 7.0 and 12.0 by using 1.0 mol/L NaOH. As shown in Fig. 4, with the increase of pH value the carvedilol adsorption amounts increased remarkably, so the maximum adsorption

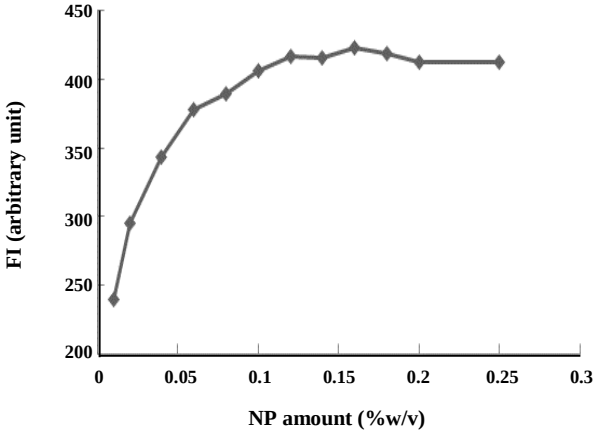


***Fig. 5: The effect of NP amount on the analytical signal for 50 ng/mL carvedilol. Conditions: pH 8.2–10; CTAB (200 ng/mL), desorption with 2 mL of MeOH, other conditions have been mentioned in the text.***

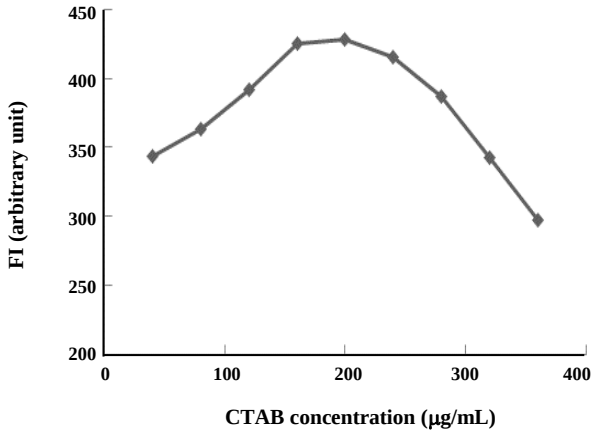


***Fig. 6: The effect of CTAB amount on the analytical signal for 50 ng/mL carvedilol. Conditions: pH 8.2–10; NP (0.14%), desorption with 2 mL of MeOH, other conditions have been mentioned in the text.***

was achieved at pHs between 8.2 to 10.0, then decreased gradually at higher pH values. The surface charge of bared MIONPs is neutral at the pH ca. 6.0 ($pH_{zpc}$ = pH zero point charge) [25,41]. Therefore, it is concluded that the surface of these particles has a negative charge when pH of the solution is above its pH ZPC. Oppositely, the surface of MIONPs is positively charged. The negatively charged surface of MIONPs was favorable for the adsorption of cationic surfactants and thus targeted analyte. On the other hand, the $pK_A$ value for carvedilol is 7.5 [15], so carvedilol is chiefly neutral at pHs higher than this value, hence has higher tendency for the extraction in hydrophobic chains of CTAB surfactant. According to these explanations, pH range of 8.2-10 was chosen for all subsequent experiments and 100 µL of 1 mol/L NaOH was used for the pH adjustment in this range.

### *The amounts of $Fe_3O_4$ NPs*

MNPs have been used as sorbents for their high surface areas and strong magnetism, compared to ordinary sorbents, so satisfactory results can be achieved with fewer amounts of NP sorbents [34]. The influence of MNPs content was studied by adding different amounts of 1% w/v $Fe_3O_4$ suspension, ranging from 0.1–2.5 mL. As can be seen in Fig. 5, the fluorescence intensities increased with the increase of magnetic adsorbents amount. When the amount of adsorbents was above 0.12% w/v, the fluorescence intensities reached the maximum and then kept invariant. According to these results, 1.4 mL from 1% $Fe_3O_4$ suspension (eq. to final concentration of 0.14%) was selected as the final amount of magnetic adsorbents used in the following studies.

### *Effect of surfactant type and amount*

High extraction efficiency was achieved when CTAB coated MIONPs were used for the extraction of carvedilol. The outer surface of hemimicelles is hydrophobic whereas that of admicelles is ionic, which provides different mechanisms for retention of organic compounds and both are suitable for the SPE method. In mixed hemimicelles phase, the adsorption is driven by both hydrophobic interactions and electrostatic attraction because of the formation of hemimicelles and admicelles on the surface of mineral oxides [27,29].

It must be mentioned that carvedilol is hardly absorbed on the surface of MIONPs in the absence of CTAB. This may be explained that the MIONPs surface without any modification is hydrophilic and has low adsorption affinity for the compounds [24]. In this study, CTAB was added to the solution at concentrations lower than its CMC (*e.g.* $1\times10^{-3}$ mol/L) [40] to modify the surface of MIONPs. From Fig. 6, it can be seen that the fluorescence intensities increased with the increasing amount of CTAB. Maximum extraction recoveries was obtained when CTAB amounts was in the range of 160-240 µg/mL. When CTAB amount was above this range,

the fluorescence of extracted analyte decreased gradually. This may be attributed that the CTAB molecules began to form micelles in the bulk aqueous solution. The formed micelles in aqueous solution caused the analyte to redistribute into the solution again [29,34]. Given these findings, 1 mL of 2000 µg/mL CTAB solution (eq. to the final concentration of 200 µg/mL) was selected as the optimum amount of CTAB in the next studies.

***Extraction and desorption time***

Adsorption and desorption time are key parameters affecting the complete extraction and recovery of the analyte, respectively. These variables were studied in the range of 3 to 30 min while the other parameters were fixed constant. The results indicated that the adsorption of carvedilol onto MIONPs and it's desorption was time dependent. The optimum fluorescence intensities were obtained when the adsorption and desorption time were 15 and 5 min, respectively. At higher times the analytical signals were approximately constant, thus these times were selected as the best adsorption and desorption time for the following experiments. The high surface area of MNPs along with homogeneous distribution of the nanosorbent throughout the sample and its super paramagnetic properties could be the possible reasons for achieving such a fast extraction process [25,29,36,40].

***Desorption condition***

Organic solvents can rapidly and completely disrupt the mixed hemimicelles and therefore the analyte is removed from the surface of NPs [24,26,27]. A variety of elution solvents including MeOH, EtOH, acidified (Ac) MeOH or EtOH, acetone (AC) and acetonitrile (ACT) was tested to evaluate the complete disruption of hemimicelles and admicelles. From Fig. 7, we found that MeOH and EtOH both had the best ability for the desorption of carvedilol from CTAB coated MIONPs. This may be attributed to proper ability of these solvents to disrupt the mixed hemimicelles or better dissolution of carvedilol. Finally, the MNPs were eluted in one or two elution cycles with MeOH and different volumes including 2.0, 5.0 and 2×2.0 mL, respectively. It was found that the elution with 2 mL volume of MeOH was sufficient for quantitative elution of the retained analyte.

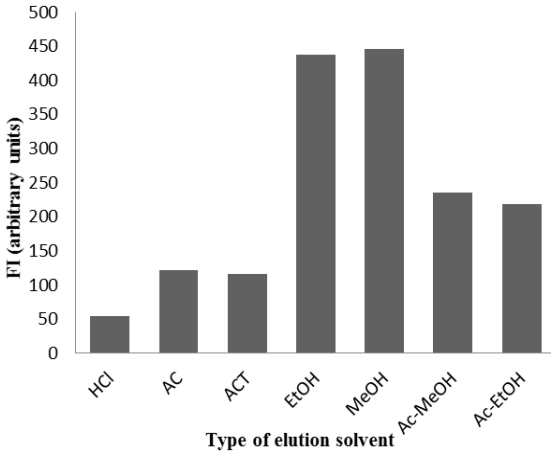


***Fig. 7: The effect of the elution solvents on the analytical signal for 50 ng/mL carvedilol. Conditions: pH 8.2–10; NP (0.14%), CTAB (200 ng/mL), desorption with 2 mL of each solvent, other conditions have been mentioned in the text.***

***Method validation***

Under the optimal experimental conditions the analytical performance of proposed MSPE method was obtained from spectrofluorimetric measurement at 354 ± 3 nm when excited at 241 ± 3 nm. The method was validated considering the linearity, sensitivity, precision, accuracy and matrix effect (ME) in order to demonstrate the feasibility of the present approach for the determination of carvedilol in plasma. Linearity of the method was established on standard aqueous solutions or spiked plasma samples and analyzed using the described extraction procedure in the range of 2–125 ng/mL. The LOD and LOQ were defined as 3Sb/m and 10Sb/m, respectively, where $S_b$ is the standard deviation of the blank measurements and m is the slope of the calibration line. The analytical figures of merit of the proposed method are summarized in Table 1.

The precision and accuracy were assessed under the optimized conditions for both intra- and inter- days and according to guidelines set by the FDA [43]. In order to do this, Quality Control (QC) samples were prepared at three concentration range (*e.g.* low, medium and high) and analyzed on the same day, 5 replicates (intra-day assay) and by duplicate analyzing, during three consecutive days (inter-day assay). Precision was expressed as the percentage Relative Standard Deviations (RSD, %) and accuracy was expressed as the relative error (R.E).

***Table 1: Analytical characteristics of the proposed method.***

| Sample | Concentration range (ng/mL) | Regression equation (n=3) | r | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|---|---|---|
| Plasma | 2.0–125.0 | 9.2313 (±0.210) C +17.411 (±0.327) | 0.9989 | 0.67 | 2.24 |
| Water | 2.0–125.0 | 9.7242 (±0.235) C + 9.068 (±0.176) | 0.9991 | 0.64 | 2.13 |

*C = carvedilol concentration (in ng/mL)*

***Table 2: Intra– and inter–day precisions and accuracies for determination of carvedilol***

| Sample | Nominal C* (ng/mL) | Precision (RSD%) (n = 6) | | Accuracy (R.E%) | |
|---|---|---|---|---|---|
| | | Intra–day | Inter–day | Intra–day | Inter–day |
| Water | 5.0 | 6.4 | 7.0 | +4.32 | +5.51 |
| | 50.0 | 1.4 | 4.4 | –2.20 | –3.26 |
| | 100.0 | 1.3 | 1.0 | +1.20 | +0.06 |
| Plasma | 5.0 | 4.2 | 4.5 | +4.26 | –0.22 |
| | 50.0 | 1.0 | 1.1 | –1.12 | –0.62 |
| | 100.0 | 0.6 | 0.5 | +0.86 | –0.88 |

**C = concentration, R.E = relative error*

The precision at each concentration level from the nominal concentration was expected to be not greater than 15% and the accuracy to be within ±15% as per US FDA guidelines [43]. Thus, good precisions were achieved with RSD values lower than 7.0% for intra-day and 6.4% for inter-day precision (Table 2). The accuracy was better than 5.5%. These results (summarized in Table 2), indicate that the method met the requirements of a bioassay [5,6,15,21,43, 45].

Also, Table 3 compares the characteristic data of the present method with those reported in the literature. The most significant feature of the proposed method is that the LOD achieved using the proposed method is better than those obtained with sensitive methods such as HPLC [15,21,44], and HPLC–MS [5] and comparable to those obtained with other sensitive methods [3,20,45]. It is also evident that the dynamic linear range, precision and recoveries achieved using the proposed method are comparable to those achieved using other methods.

***The recovery experiment and interference study***

Accuracy is also calculated as the assay's percentage recovery of a known added amount of analyte in a sample (spiked recovery approach). For this purpose, the QC samples were spiked with carvedilol at the concentration of 5, 50 and 100 ng/mL, respectively. For each concentration level, three repeated experiments were made and the mean values were taken. The obtained recoveries were summarized in Table 4 and ranged from 94.4% to 100.7%, which seemed to be satisfactory.

As well as, in order to evaluate the matrix effect on the analytical response of carvedilol, the slopes of calibration graph obtained with spiked plasma samples were compared with those obtained with solvent-based standards. For this purpose the matrix-to-solvent slope ratio was calculated and found to be 0.95. This result showed that the matrix does not influence the extraction efficiency.

Also, typical spectra for carvedilol standard solution, blank plasma, a plasma sample obtained from one volunteer, and the later spiked with carvedilol are shown in Fig. 3. No additional peaks, caused by interfering compounds, were observed at the used emission wavelength in this work. Therefore, the similarities in the excitation and emission spectra for each sample, along with reasonable recoveries indicated that there was not any significant matrix effect in this work.

***The application of the method***

The recommended methodology was successfully applied for the extraction and spectrofluorimetric determination of carvedilol in real plasma samples.

***Table 3: Analytical characteristics of different methods used for extraction and determination of carvedilol.***

| Ex./determination Method | Sample | Concentration range (ng/mL) | r | RSD% | LOD (ng/mL) | Mean recovery (%) | Ref. |
|---|---|---|---|---|---|---|---|
| LLE/HPLC-MS | S | 0.08-50 | 0.9972-0.9997 | 3.5-9.1 | 0.08(LLOQ) | 98.2-106 | 2 |
| VA-LLLME/HPLC & CE | U & P | 5-1000 | 0.9998 | 2.2-9.1 | 0.20 | 97.1-108 | 3 |
| SPE/HPLC–MS-MS | U | 25-200 | 0.9992 | 2.0-5.8 | 4.73-8.07 | 84.3-97 | 5 |
| SPE/UPLC-MS-MS | P | 0.05-50 | 0.9995 | 0.7-3.9 | 0.015 | 94-99 | 6 |
| DLLME/HPLC | P | 20-1000 | 0.9990 | 3.1-9.7 | 6.0 | 93-99 | 15 |
| SBSE/HPLC | S | 1-120 | 0.9990 | 2.4-3.2 | 0.3 | 94-103 | 20 |
| VA-LLE/MEKC | U | 5-100 | 0.9985 | 2.0-9.4 | 1.0 | 81-113 | 21 |
| On-line deproteination/HPLC | P | 1-50 | 0.9995 | 0.7-3.8 | 0.8(LOQ) | - | 44 |
| SLE/LC–MS-MS | P | 0.2-100 | 0.9980< | 11.9 | 0.2(LLOQ) | 76< | 45 |
| MSPE/F | P | 2.0–125 | 0.9989–0.9991 | 1.3-6.4 | 0.67 | 94.4-100.7 | This work |

*LLE = liquid-liquid extraction; SPE = solid phase extraction; VA-LLME = vortex–assisted LL microextraction; DLLME = dispersive LL microextraction; SLE = supported liquid extraction; SPSE = Stir bar sorptive extraction; MEKC = Micellar electrokinetic chromatography; CE = capillary electrophoresis; UPLC = ultra-performance liquid chromatography; F = spectrofluorimetry; P= plasma; U = urine.*

***Table 4: Recoveries of carvedilol from spiked plasma samples.***

| Sample | Added (ng/mL) | Found ± SD (n = 3) (ng/mL) | Recovery (%) |
|---|---|---|---|
| Plasma | 5.0 | 4.72 ± 0.32 | 94.4 |
| | 50.0 | 50.3 ± 1.29 | 100.6 |
| | 100.0 | 100.7 ± 1.64 | 100.7 |

The plasma sample for this purpose was collected after carvedilol had been ingested and treated as described in the "Procedure for plasma samples" section. The carvedilol concentration in the plasma sample was found to be 39.8 ng/mL.

## CONCLUSIONS

In this work, MSPE based on NPs combined with spectrofluorimetry was successfully developed for the extraction and determination of carvedilol in human plasma samples. In this method the sample preparation time is dramatically decreased by the fact that MIONPs dispersed in the bulk solution, and as a result, extraction can be achieved very quickly by using magnet. So, there is no need to use a centrifuge for phase separation. Compared with methods in which CE, HPLC or HPLC–MS are used to determine the target analyte, the proposed method does not require high levels of financial investment or involve high instrument maintenance costs. Method validation using spiked real samples demonstrated that the method is capable of detecting trace carvedilol with adequate accuracy and precision. Also, sensitivity of the method is enough for the determination of carvedilol in biological fluids. All these results indicated that using MSPE combined with spectrofluorimetric measurement is a very simple, safe, sensitive, rapid and inexpensive method for the extraction and determination of carvedilol.

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