

# Preconcentration and Determination of Ciprofloxacin with Solid-phase Microextraction and Silica-coated Magnetic Nanoparticles Modified with Salicylic Acid by UV-Vis Spectrophotometry

Mohsen Varak Neshin<sup>1</sup>, Mahmoud Ebrahimi<sup>1\*</sup>

<sup>1</sup> Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, IRAN

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## ABSTRACT

In the present age, the pharmaceutical application continues to increase and this results in the environmental pollution. As a result, pharmaceutical control is a common technique in many labs. This project is on the improvement of the procedure to determine a small amount of ciprofloxacin (CF) in bio-environmental sample. Regarding the fact that each method requires preliminary measures to preconcentrate and prepare analyte prior to measurement and recognition. In recent study, preconcentration of samples containing CF (as antibiotic) based on solid-phase microextraction method using silica-coated magnetic nanoparticles modified with salicylic acid (SA) was proposed which acted as sorbent. In addition, UV-Vis spectrophotometry was carried out to determine the amount of CF. The wavelength of maximum absorption ( $\lambda_{max}$ ) for this drug was 275 nm. Also, the optimum conditions of various parameters such as pH of solution, amount of magnetic nanoparticles, time, the effect of stirring speed on the extraction and desorption, the rate of centrifugation, the volume of aqueous and organic-phases, temperature and salt effect were investigated. The dynamic range was obtained between 0.05-0.15 mg L<sup>-1</sup>, LOD and RSD (%) were 0.002 mg L<sup>-1</sup> and 1.878 respectively. Results showed using this procedure increased the concentration factor to 68.36. Relative recovery was achieved by spike method.

**Keywords:** ciprofloxacin, magnetic nanoparticles, solid-phase microextraction, UV-Vis spectrophotometry

## INTRODUCTION

The quinolones have emerged as one of the most important classes of antibiotics of the previous decade. CF [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperariny)-3-quinolone carboxylic acid] (**Figure 1**) is a synthetic fluoroquinolone derivative which has demonstrated broad-spectrum activity against many pathogenic Gram-positive and Gram-negative bacteria. The bacterial action of CF results from interference with enzyme DNA gyrase which is needed for the synthesis of bacterial DNA [1-4]. The drug is structurally related to different quinolones including cinoxacin, lomefloxacin, enoxacin, nalidixic acid, norfloxacin, pefloxacin and ofloxacin.

There is several interest in determining fluoroquinolones for the purpose of pharmaceutical quality control. Most of the analytical methods for the determination of CF employ high-performance liquid chromatography (HPLC). In 1998, Carlucci reported a review of the published HPLC assays that used fluorescence or UV detection [5]. Then, other works have been reported employing fluorescence detection [6-11] and UV detection [12, 13]. The HPTLC method has been developed for the determination and the purity control of CF in coated tablets [14, 15]. Other different methods were reported such as spectrophotometry [16-22], fluorimetry [22-24], capillary electrophoresis [25-32] and immunoassay [33, 34]. Both spectrophotometric [35-38] and chemiluminescent [39, 40] detection were proposed as automatic spectroscopy procedures.

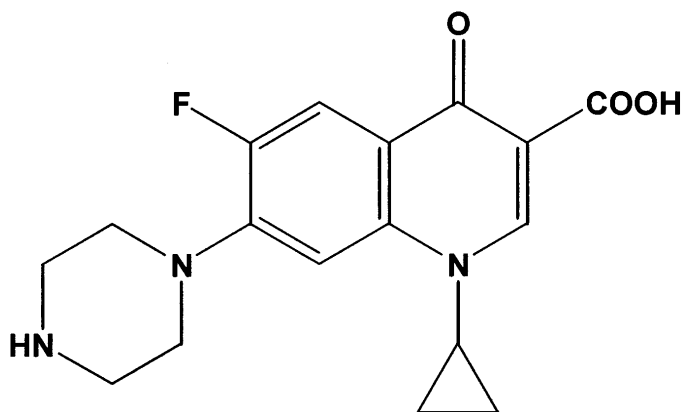


Figure 1. Structure of CF

UV-Vis spectrophotometry is considered as the most convenient analytical technique, because of its inherent simplicity and wide availability in most quality control laboratories.

Solid-phase microextraction, first introduced with Belardi and Pawaliszyn more than a decade ago [41], is a simultaneous sampling, matrix removal, preconcentration and simple technique. Solid-phase microextraction has been more and more used for sample preparations in analytical laboratories.

Magnetic nanoparticles, a new kind of nanometer-sized material, are widely used in the fields of biomedicine, biotechnology and as an efficient adsorbent with large specific surface area and small diffusion resistance [42-55].

Several methods have been reported to synthesize magnetic nanoparticles, such as hydrothermal synthesis [56], microemulsion [57], chemical co-precipitation [58], oxidation of Fe (OH)<sub>2</sub> by H<sub>2</sub>O<sub>2</sub> [59], microwave irradiation [60], R-ray irradiation [61] and etc. Co-precipitation is the cheapest and simplest synthesis method.

However, it should be pointed out that pure inorganic magnetic nanoparticles (such as Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>2</sub>O<sub>3</sub>) can easily form extensive aggregates, which may alter their magnetic properties [51, 62]. Moreover, these are not target-selective and are unsuitable for several samples. A suitable coating is essential to overcome such limitations. To overcome latter problem, physical or chemical modification of the sorbent surface by some organic compounds, especially chelating ones, is usually used to load the surface with some donor atoms such as oxygen, sulfur, nitrogen and phosphorus [49, 51].

SA is a commercial ligand with a carboxylic and a phenolic functional group which can act as electron pair donors reacting with most of hard and intermediate cations. It has already been used, for example, as the modifier in chelating resins like Amberlite XAD-2-SA, Amberlite XAD-4-SA and silica gel-SA and it have shown nice sorption capacity [63-65].

The aim of this work was to develop an analytical method for the preconcentration and determination of CF with solid-phase microextraction and silica-coated magnetic nanoparticles modified with SA by UV-Vis spectrophotometry.

## EXPERIMENTAL

### Materials

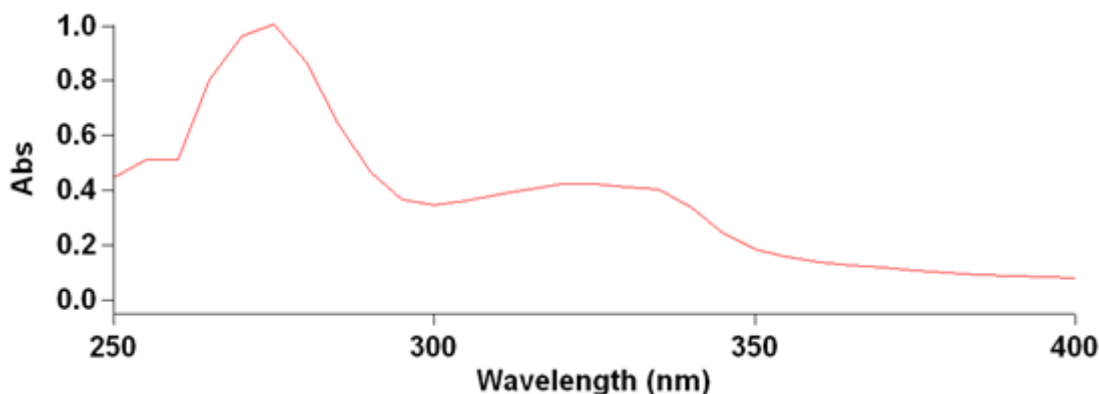
All chemicals were of analytical-reagent grade. The following materials were used in this study:

CF from Acofarme (Spain), chloroform and methanol from BDH (England), ethanol, sodium chloride, acetic acid and borax from Merck (Germany) and FeCl<sub>3</sub>.6H<sub>2</sub>O, FeCl<sub>2</sub>.4H<sub>2</sub>O and tetraethoxysilane (TEOS) from Sinopharm chemical Reagent Co. (China) were obtained. SA, glycerol and NH<sub>3</sub>.H<sub>2</sub>O were analytical grade and commercially available products. Aqueous solutions of drug were prepared with distilled water. The washed magnetic nanoparticles was stored in deionized water. Buffer solutions of required pH were made from 0.1 mol L<sup>-1</sup> NaOH Merck (Germany) solution and 0.1 mol L<sup>-1</sup> HCl Merck (Germany) solution.

### Apparatus

The following instruments were used in this study:

A single-beam model (Varian, Cary 50 Bio, Australia) UV-Vis spectrophotometer equipped with 1 mm matched quartz cells was used for absorbance measurements. A Metrohm model (780, Switzerland) pH-meter with a combined glass electrode, libra model (Sartorius, GC1603P, Germany) with an accuracy of ± 0.0001, ultrasonic



**Figure 2.** Absorption spectra of CF after solid-phase microextraction

processor model (Hielscher, UP100H, Germany) and centrifuge model (Dynamica Velocity, 18R, Austria) were used. Stirring of the solutions was carried out with a (Heidolph MR 3001, Schwabach, Germany) magnetic stirrer. Infrared spectra were recorded by Fourier transform infrared spectrometer (FT-IR, Perkin Elmer, spectrum 100). Scanning electron microscopy (SEM) was performed to measure the magnetic nanoparticles size and shape (SEMEDX, XL30, Philips, Netherland). X-ray diffractometer (XRD) (38066 Riva, d/G.Via M. Misone, 11/D (TN) Italy) was used.

### Preparation of Silica-coated Magnetic Nanoparticles

The magnetic nanoparticles were prepared by the conventional co-precipitation method with minor modifications [44]. For this method, ultrasonic processor with an ultrasonic bath was used instead of magnetic stirring.  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (11.68 g) and  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (4.30 g) were dissolved in 200 mL deionized water under nitrogen gas in an ultrasonic bath at 85°C for small minutes leading to smaller and more homogenized particles. 20 mL of 30%  $\text{NH}_3 \cdot \text{H}_2\text{O}$ , was added to the solution. The color of bulk solution changed from orange to black fastly. The magnetic precipitates were washed twice with deionized water and once by 0.02 mol  $\text{L}^{-1}$  sodium chloride. The washed magnetic nanoparticles was stored in deionized water at a concentration of 40.0 g  $\text{L}^{-1}$ .

Then, the suspension prepared above (20 mL) was placed in a 250 mL round-bottom flask and allowed to settle. The supernatant was removed, and an aqueous solution of [TEOS, 10% (v/v), 80 mL] was added, followed by glycerol (60 mL). The pH of the suspension was adjusted to 4.5 using glacial acetic acid, and the mixture was then stirred and heated at 90°C for 2 h under a nitrogen atmosphere. After cooling to room temperature, the suspension was washed sequentially by deionized water ( $3 \times 500$  mL), methanol ( $3 \times 500$  mL) and deionized water ( $5 \times 500$  mL). The silica magnetic composite was stored in deionized water at a concentration of 40.0 g  $\text{L}^{-1}$ .

### Preparation of Silica-coated Magnetic Nanoparticles Modified with SA

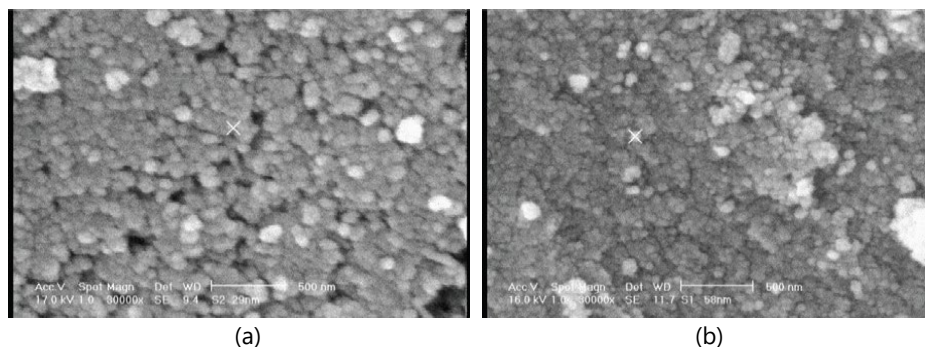
25 mL of silica-coated magnetic nanoparticles prepared as described above was washed with ethanol ( $2 \times 100$  mL) and then diluted to 150 mL with 3.3% SA solution and 16 mmol  $\text{L}^{-1}$  acetic acid solution (pH 4.5). The solution was transferred to a 500 mL 3-necked round-bottom flask and later stirred and heated at 60°C for 2 h under a nitrogen atmosphere. The resulting magnetic nanoparticles were washed with deionized water three times and twice by methanol, then dried into powders at room temperature under vacuum.

## METHOD

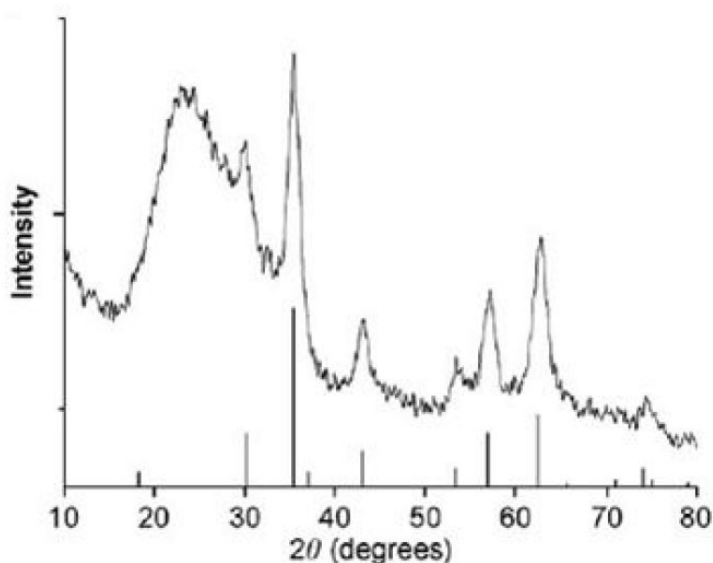
The solution was made 0.4 mg  $\text{L}^{-1}$  of CF in chloroform. The UV-Vis spectrophotometry absorption was used and the absorbance was 0.098 at 275 nm. This value was the base of analytical comparison of CF before and after microextraction. After the microextraction with magnetic nanoparticles and at the same concentration, absorbance of CF rose to 1.005 (Figure 2).

To prepare a stock solution, 50  $\mu\text{g}$  CF was added in to the flask 50 mL and dissolved in distilled water. The pH of the solution with 0.1 mol  $\text{L}^{-1}$  NaOH was raised to 10.5. Then the traces of borax was added and a 1 mg  $\text{L}^{-1}$  solution of CF was made.

In this method, after preparation of the solution 0.4 mg  $\text{L}^{-1}$  from CF of stock solution in pH suitable at aqueous-phase, magnetic nanoparticles was added to solution and magnetic stirring was set about 10 min. Then it centrifuged for 5 min. After this step, solution was discarded and magnetic nanoparticles with absorbed CF attached to the magnet. 3 mL of chloroform was added. Stirring for 10 min was set to be stirred by magnets and



**Figure 3.** SEM images of synthesized magnetic nanoparticles (a) and modified magnetic nanoparticles (b) [45]



**Figure 4.** XRD pattern of silica-coated magnetic nanoparticles [45]

later centrifuged for 5 min. The absorption spectrum of the chloroform solution of CF after the desorption process by UV-Vis spectrophotometry was recorded. The absorbance at 275 nm was 1.005, it was reduced due to the high absorption of CF concentration. Several tests and low concentrations of CF solution, containing a concentration of 0.15 mg L<sup>-1</sup> was found suitable for this solution. After solid-phase microextraction steps as above, the absorption spectra of the solution by UV-Vis spectrophotometry was 0.210.

## RESULTS AND DISCUSSION

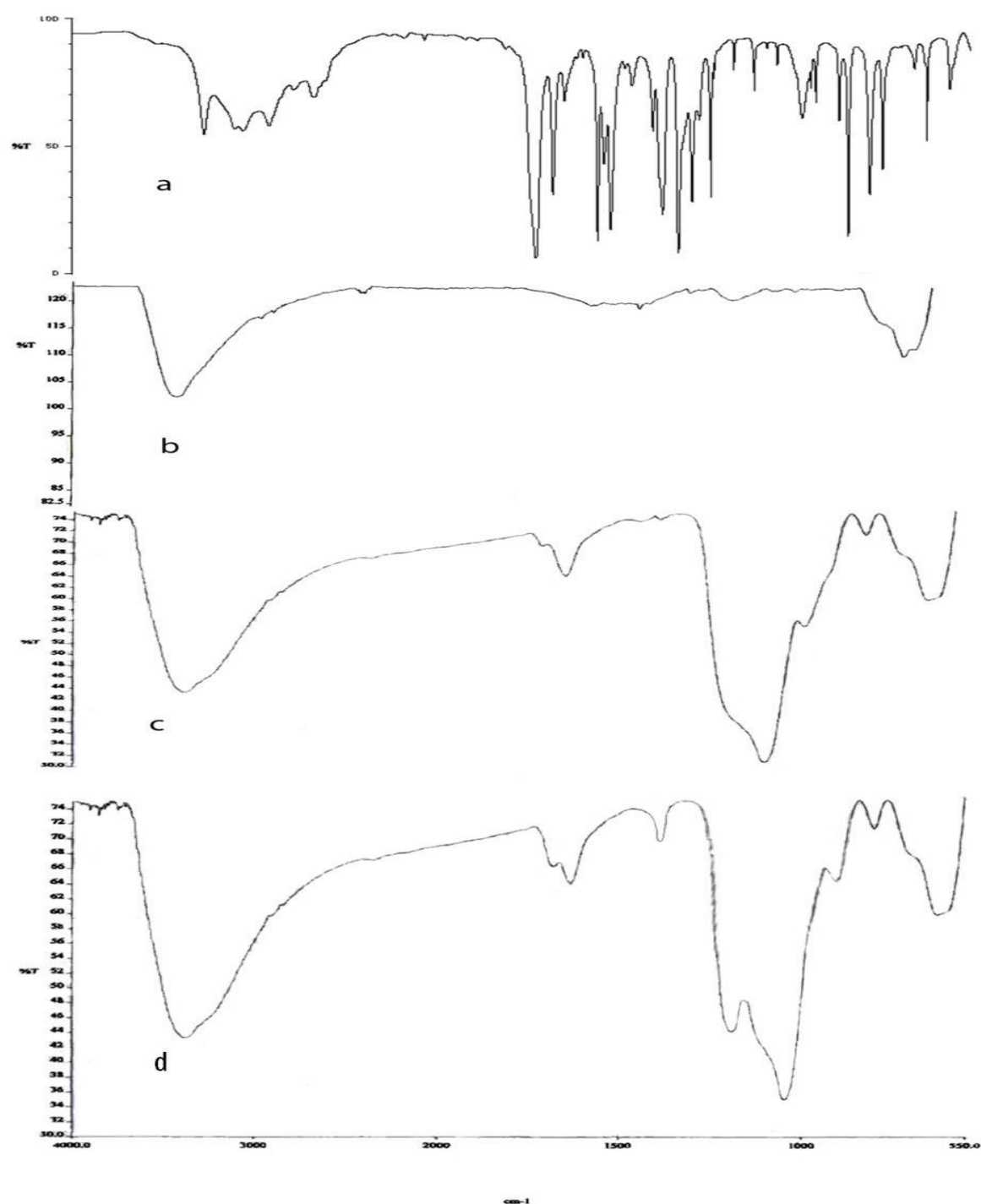
### Characteristics of Modified Magnetic Nanoparticles

The surface and textural morphology of silica-coated magnetic nanoparticles with SEM image is illustrated in **Figure 3**. As shown in **Figure 3**, the pristidin magnetic nanoparticles had a mean diameter of 29 nm. Using the ultrasonic processor caused smaller and more homogenized magnetic nanoparticles prepared [66]. After modification process, the modified magnetic nanoparticles prepared are in the range of 58-73 nm in diameter. This shows that the magnetic nanoparticles have been completely coated with the silica and SA.

The typical XRD profile of silica-coated magnetic nanoparticles is shown in **Figure 4**. The broad peak at around  $2\theta = 20^\circ$  in the XRD pattern is due to the amorphous silica shell on the surface of the magnetic nanoparticles.

The FT-IR spectrum of silica-coated magnetic nanoparticles modified by SA has prominent bands at 1680 cm<sup>-1</sup>, 1486 cm<sup>-1</sup> and 1387 cm<sup>-1</sup> due to carboxylate, OH (bending) and phenolic group vibrations, respectively (**Figure 5**).

All parts of magnetic nanoparticles production and related figures obtained from paper [67].



**Figure 5.** FT-IR spectra (a) SA (b) magnetic nanoparticles (c) SiO<sub>2</sub> coated magnetic nanoparticles (d) SiO<sub>2</sub> coated magnetic nanoparticles modified with SA [45]

### Spectral Characteristic

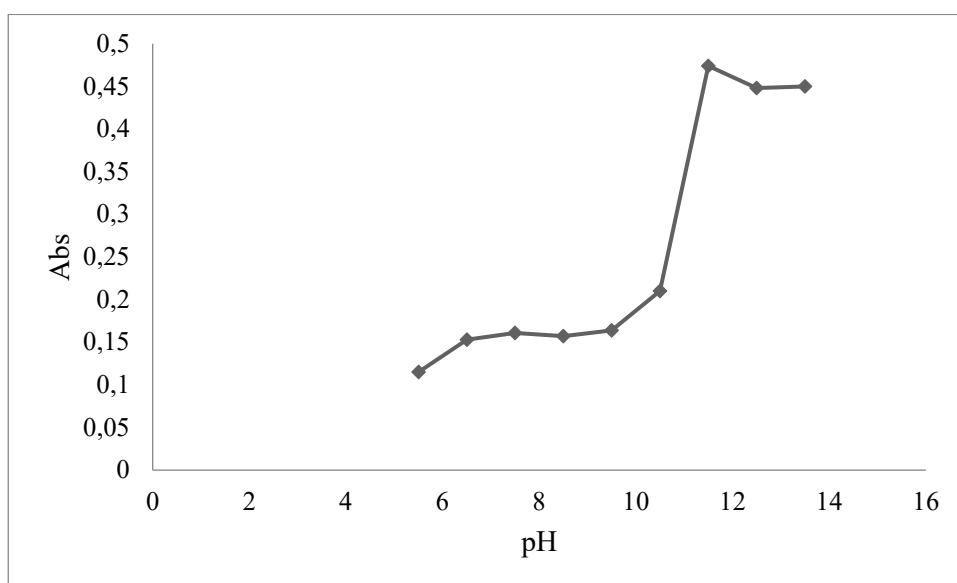
According to the studies in the literature, absorption of CF occurs between 250 to 300 nm wavelength. The wavelength of maximum absorption ( $\lambda_{\max}$ ) of this drug is 275 nm that is found experimentally.

### Effect of Experimental Variables

The optimal conditions for solid-phase microextraction, all parameters and procedures were studied and tested in this research. In the entire optimization process for the solution of CF, concentration was 0.15 mg L<sup>-1</sup>. The

**Table 1.** The optimized parameters and maximum absorbance

Parameter	Amount	Abs <sub>(max)</sub>
pH	11.5	0.474
Amount of magnetic nanoparticles (mg)	2	0.511
Extraction time (min)	10	0.511
Centrifuge Time (min)	20	0.516
Desorption time (min)	10	0.518
Stirring speed on the extraction process (RPM)	800	0.570
The rate of centrifugation (RPM)	5000	0.571
Stirring speed on the desorption process (RPM)	500	0.573
Volume of aqueous-phase (mL)	20	0.674
Volume of organic-phase (mL)	3	0.676
Temperature (°C)	55	0.838
Salt effect (w/v)%	1	0.887

**Figure 6.** Effect of pH on absorption in the extraction process

optimized parameters and maximum absorbance are set in [Table 1](#). In the following, the curves of some important optimized parameters are reported.

### The optimum pH in the Extraction Process

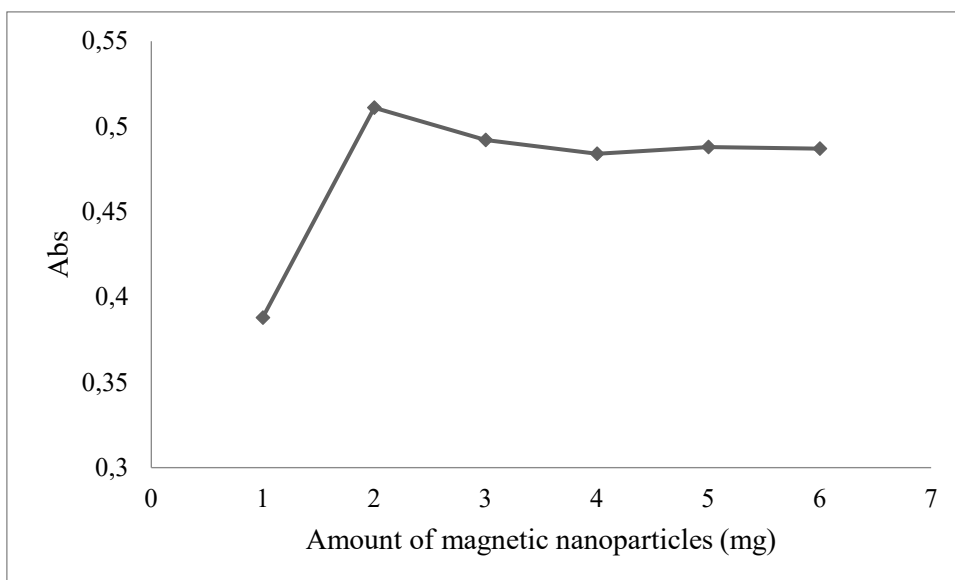
pH plays an important role in the extraction. The effect of pH on extraction was studied. Therefore, pH of 5.5 to 13.5 were tested and the results obtained at pH 11.5 was the highest absorbance. This drug has a positive charge at alkaline solution and due to the fact that  $pH_{zpc}$  at pH 7 is neutral so the maximum interaction between the analyte and the magnetic nanoparticles observed at pH 11.5 ([Figure 6](#)).

### Optimization of the Amount of Magnetic Nanoparticles

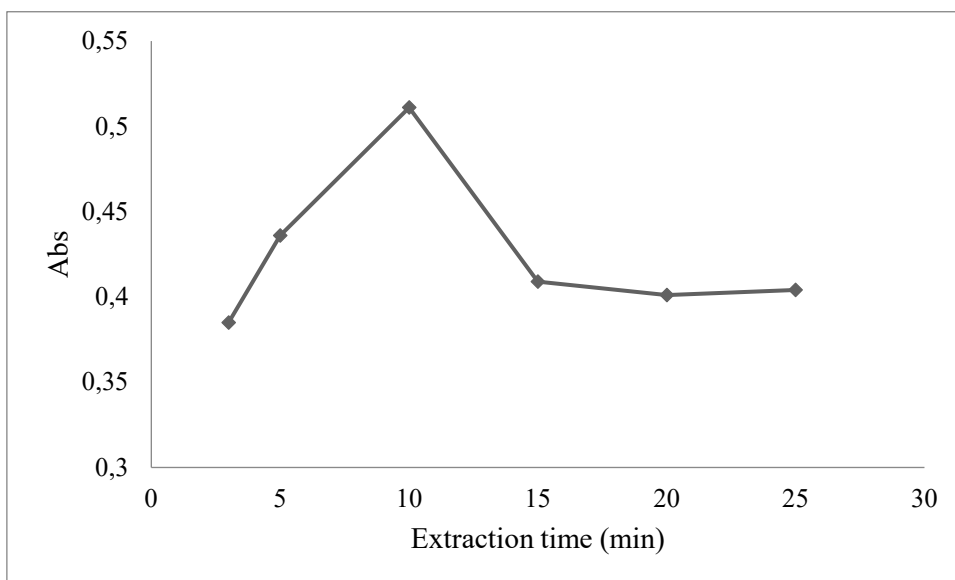
To optimize the amount of magnetic nanoparticles, different doses (1, 2, 3, 4, 5 and 6 mg) were tested and it was found that for 2 mg maximum absorption is achieved. Less than 2 mg, extraction efficiency is low because the amount of magnetic nanoparticles is not enough for extraction and cannot uptake all of the amount of analyte molecules on the magnetic nanoparticles. More than 2 mg, increasing the amount of magnetic nanoparticles does not affect the extraction efficiency ([Figure 7](#)).

### Optimization of the Extraction Time

Extraction time affects the equilibrium conditions. In aqueous solution analyte molecules should have enough time to transfer to the magnetic nanoparticles. The optimization time of 3, 5, 10, 15, 20 and 25 min were analyzed and the conclusion was that by 10 min extraction the highest absorption is achieved. After 10 min, the extraction



**Figure 7.** Effect of different amounts of magnetic nanoparticles on absorption



**Figure 8.** Influence of different extraction times on absorption

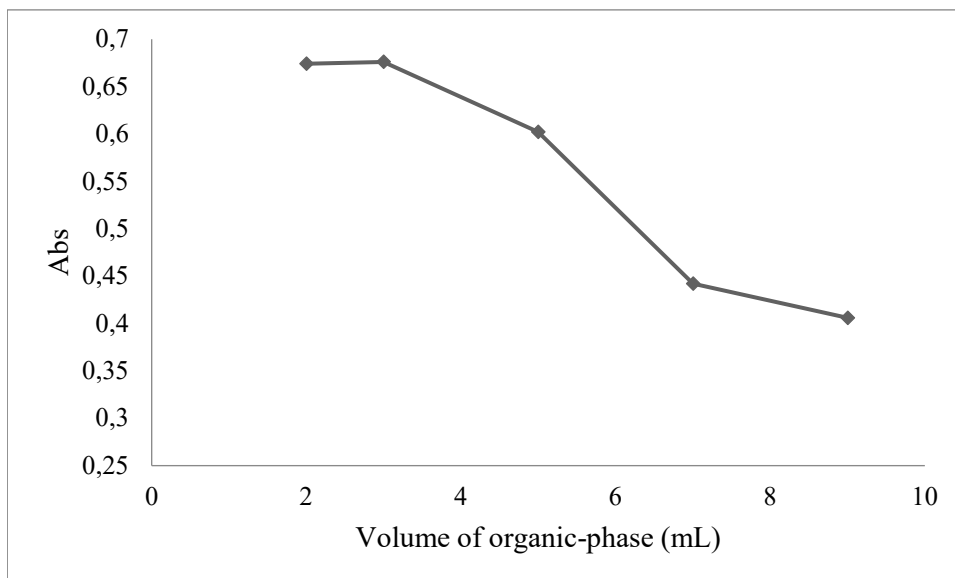
efficiency decreases which may be because all the adsorbed analyte molecules are re-entered to aqueous solution due to increasing time (Figure 8).

### Optimization of the Volume of Organic-phase Solvent in the Desorption Process

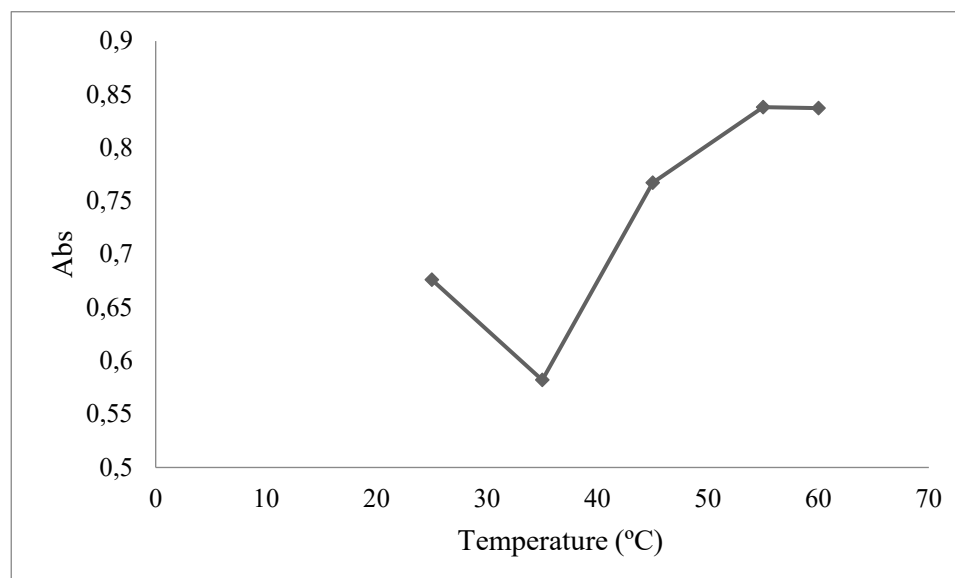
The complete extraction of the analyte depends on the organic-phase solvent volume. Optimization was done in 5 different volumes. At higher volumes, the extraction efficiency is reduced. The main reason of reduced extraction efficiency with the increased volume of organic-phase solvent is analyte dilutions. Also, with increasing the volume of organic-phase, the analyte in the adsorbent surface decreases. The best result was obtained by 3 mL of organic-phase volume. (Figure 9).

### The Optimum Temperature in the Extraction Process

Setting the temperature to complete the reaction is important and a suitable temperature for the reaction should be considered. To optimize the temperature, the extraction process was carried out at several temperatures. At 35°C the extraction efficiency reduced. At higher temperatures, as the temperature increases the speed of transition of



**Figure 9.** Effect of different volumes of organic-phase solvent on absorption



**Figure 10.** Effect of different temperatures on absorption in the extraction process

analyte molecules to the magnetic nanoparticles increases and the extraction efficiency rises. Then, it was concluded that the optimum temperature, is 55°C (**Figure 10**).

### Analytical Parameters

The calibration curve for the sample treated according to the proposed procedure is linear for the concentration range 0.05-0.15 mg L<sup>-1</sup> (**Figure 11**). The analytical parameters are summarised in **Table 2**.

### Analytical Application

For real sample project, the hospital effluent was taken from Razavi hospital (Razavi Khorasan, Mashhad) and analyzed.

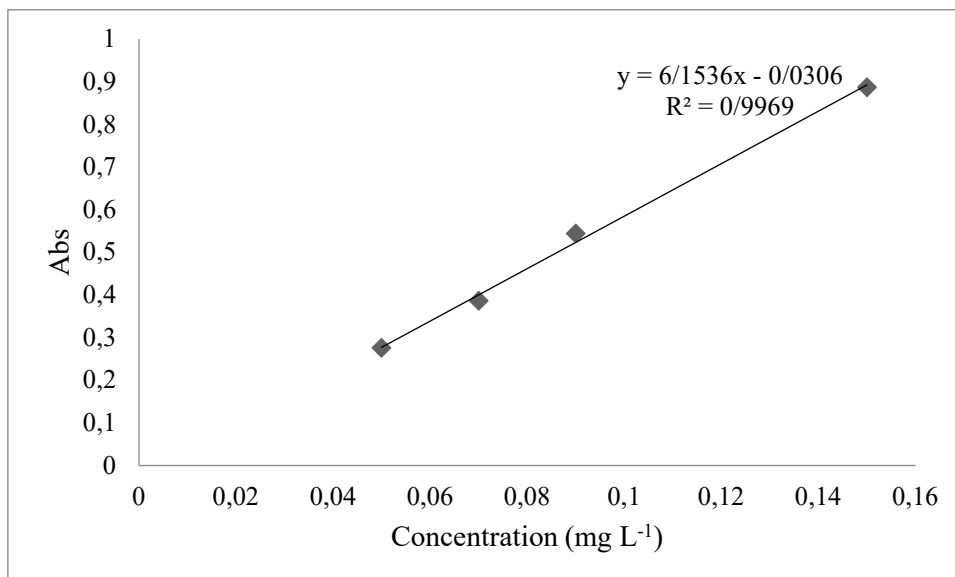
There was no CF in hospital effluent, so we added 0.07 mg L<sup>-1</sup> drug to it and then relative recovery was done by spike method.

The relative recovery of CF was done by comparing the absorbance values obtained of the pure drug with absorbance obtained from the spiked method.



**Table 2.** Analytical parameters

Parameter	
Linear dynamic range (mg L <sup>-1</sup> )	0.05-0.15
Detection limit (mg L <sup>-1</sup> )	0.002
RSD (%)	1.878
Concentration Factor	68.36

**Figure 11.** Calibration curve for this method

Absorbance values obtained at 0.07 mg L<sup>-1</sup> for the pure drug, after solid-phase microextraction and its optimum value was 0.386. Absorbance obtained after solid-phase microextraction in optimal conditions for solution made to the spike method at the same concentration was 0.357. Relative recovery was 92.48%.

## CONCLUSION

The results of the experiments show that the method of solid-phase microextraction with silica-coated magnetic nanoparticles modified with SA is a suitable extraction method for preconcentration and measurement of small quantities CF. The method of microextraction uses small amounts of magnetic nanoparticles (2 mg) that after optimization steps, is able to detect minor amounts of CF. These magnetic nanoparticles have relatively high adsorption as compared to the similar materials because of their smaller size. The size of the produced magnetic nanoparticles was determined by XRD analysis and SEM. Also, magnetic nanoparticles can be separated from the aqueous solution easily. The measurement of these drugs using UV-Vis spectrophotometry is done precisely. The benefits of this method are low detection limit, high levels of accuracy, concentration factor and relative recovery. This method is very fast, easy and efficient for a wide variety of analytes. In general, the discussed method is a reliable technique for measuring drugs in environment and other real samples.

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