Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 3 Issue 4 April 2019

Research Article

Magnetic Solid-Phase Extraction Using Fe_3O_4@SiO_2 Hybrid Nanosorbents for the Uptake of Ciprofloxacin in Biological Samples

Mozhdeh Mardani and Zarrin Es'haghi*

Department of Chemistry, Payame Noor University, Iran

***Corresponding Author:** Zarrin Es'haghi, Department of Chemistry, Payame Noor University, Iran.

Received: January 11, 2019; **Published:** March 12, 2019

Abstract

An efficient and simple method has been developed for the quantitative determination of ciprofloxacin in biological samples. The method is based on the measurement of ciprofloxacin in the presence of magnetic Fe_3O_4 nanoparticles functionalized with sodium hexafluorosilicate using UV-Vis spectrophotometer. Magnetic Fe_3O_4/SiO_2 nanoparticles were synthesized via co-precipitation method. Detection was performed at 275 nm, the maximum wavelength of ciprofloxacin. In this condition, linear range was achieved in two areas. For low concentrations area 0.1 to 1 and high concentrations areas 1-20 (mg/L), were evaluated. The method limits of detection and qualification were 0.0472 and 0.157 (mg/L) for low concentrations area and 0.458, 1.52 (mg/L) high concentrations, with relative standard deviations (RSD) values less than 5% (n = 6) respectively. The method was validated through the parameters of linearity, accuracy, precision, limit of detection, limit of quantitation. Also in optimal conditions, the concentration of ciprofloxacin in real samples of blood were determined and relative recoveries for real samples were evaluated too.

Keywords: Solid Phase Extraction; Ciprofloxacin; Magnetic Fe_3O_4/SiO_2 Nanoparticles

Introduction

Recently, antibiotics became more important as focus point of research due to their high amounts in the environment and the increasing bacterial confrontation. Fluoroquinolones (FQs) are piperazinyl derivatives of quinolones, and are largely used to treat humans and food making animals, due to their broad-spectrum activity against the bacteria [1]. Among fluoroquinolones, norfloxacin (NOR) and ciprofloxacin (CIP) are the most widely used antibiotics [2]. Ciprofloxacin(1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylicacid) is a 4-quinolone derivative, derived from nalidixic acid [3]

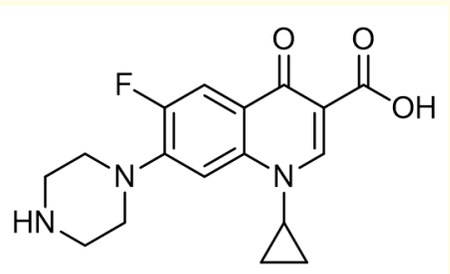

Figure 1: Molecular structures of ciprofloxacin.

It provides effective treatment for a variety of infections particularly those of the urinary tract, respiratory tract, gastrointestinal tract, skin and soft tissues [4].

It is very important to determine ciprofloxacin for the purpose of pharmaceutical quality control. The literature survey revealed that the most methods for determination of ciprofloxacin in tablets and injection formulations include separation techniques [5-14], electrochemical procedures [15-17], spectrophotometric and spectrofluorimetric methods [18-29]. Also, several methods have been reported for the quantitative analysis of the ciprofloxacin, such as titrimetry [30], microbiology [31], HPLC [32] and AAS [33,34]. Furthermore, the reduction process of iron (III) to iron (II) by ciprofloxacin was used as the basis for its quantification based on a colorimetric method [35].

Spectrophotometry method is significant once they can be used in the simple and small research laboratory which there are not expensive instrumentations. The present work aims to develop a highly sensitive, simple and rapid UV-Vis spectrometric method for application in quality control analysis. This study has been offered a solid phase extraction method based on functionalized magnetic nanoparticles. This method is simple, fast and affordable to extract

ciprofloxacin from aqueous solution. Synthesis of functionalized magnetic nanoparticles of iron and sodium hexafluorosilicate is done with the help of co-precipitation method.

Materials and Methods

Reagents

The drug ciprofloxacin was donated by Daroopakhsh pharmaceutical (Tehran, Iran). Methanol, ethanol, and nitric acid were supplied from Merck (Darmstadt, Germany) as well as, all the other compounds. The drug was dissolved in methanol to obtain a concentration of 500 mgL^{-1} for the stock solution. The standard solutions and QC samples were made from the stock solution by diluting with deionized water.

Apparatus

UV-Vis Spectrophotometer, model T80 (PG Instruments, UK), was used for recording absorbance spectra. The synthesized nanocomposites were washed and separated from the solution by Milli-Q water. An Ultrasonic device, model UP 400S, made in Germany was used for functionalization and dispersion of mesoporous MWCNTs. A pH meter, (model 827 pH lab) Metrohm, Swiss was used for adjusting the pH of solutions.

Preparation of Fe3O4/SiO2 nanoparticles

Fe_3O_4/SiO_2 NPs were produced through two steps. First, Fe_3O_4 NPs were prepared by co-precipitation method as a convenient way. Fe_3O_4 NPs was synthesized by dissolving $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ in water with ratio of 2:1. 12.5 mL solution of ammonia (28%) was dropped slowly into the mixture solution of the two salts, until the system is solidified due to the formation of Fe_3O_4 particles. The precipitate was separated by a magnet and was rinsed with water and then ethanol several times, followed by drying at 60°C.

In the second stage, the Fe_3O_4 nanoparticles were added to the sodium fluorosilicate solution (Na_2FSi_6). After an hour stirring, solution fixed for 6 hours at 25°C. The precipitate was separated by a magnet and was rinsed with water and then ethanol several times, followed by drying at 60°C for 2 days.

Solid-phase extraction procedure

5 ml standard solutions of ciprofloxacin (10 mg/L) was added to a vial containing 5mg Magnetic Fe_3O_4/SiO_2 nanoparticles. The solution was stirred for 5 min to complete the extraction process at room temperature. Afterward, the vial was placed on a strong magnet and then the suspension had become clear after 5 min was elapsed since the NPs were attracted to a magnet. Consequently, the supernatant solution could be decanted.

Finally, 3 ml ethanol as adsorption solvent was added to the sorbent. The mixture was stirred for 10 min and nanoparticles were separated by an external magnet. The sorbent was eluted by desorption solvent, and the desorbed solution was transferred to the UV-Vis for measurement of its absorbance at the maximum wavelength of ciprofloxacin (275 nm).

Adsorbent characterization

The FTIR spectra of Fe_3O_4 and Fe_3O_4/SiO_2 NPs are compared in (Figure 2). The absorption peaks at 586, 1620 and 3413 cm^{-1} appeared in the two FTIR spectra, were correspondent to the Fe-O vibration related to the magnetite phase, bending mode and the stretching mode of the –OH group, respectively [36]. In FTIR spectra of the Fe_3O_4/SiO_2 NPs (curve ii), the absorption intensity of Fe–O group declined with the addition of silica percentage and a strong absorption signal of the Si–O–Si group appeared at 1080 cm^{-1} that demonstrate the covering of silica on the magnetite surface [37].

Figure 2: The FT- IR of Fe3O4 (i) and Fe3O4/SiO2 (ii).

The crystalline structures of the Fe_3O_4 and Fe_3O_4/SiO_2 nanocomposites analyzed using XRD (Figure 2). For Fe_3O_4 NPs (curve i), the presence of six characteristic diffraction peaks at 2θ=30.24, 35.63, 43.28, 57.29 and 62.86° indicated that magnetite had a cubic spinel structure [38]. The characteristic peaks for Fe_3O_4/SiO_2 NPs (curve ii) were similar to that of Fe_3O_4 NPs, which demonstrated that the modification of the surface MNP with silica layer had no effect on the stability of the crystalline phase of Fe_3O_4 NPs. The lack of SiO_2 characterized peaks demonstrated that the coated SiO_2 were mainly amorphous. However, the existence of SiO_2 coating had been con-

firmed due to the decrease in the XRD diffraction peak intensities of Fe_3O_4 NPs.

Figure 3: The XRD of Fe3O4(a) and Fe3O4/SiO2(b).

Blood Samples treatment

Blood samples for evaluation of the procedure were obtained from Ghaem hospital of Mashhad, Iran. The samples were selected from 30-45 years old men. Serum should be clear and free from all red cells. For serum, allow the blood to clot sixty minutes and separate by centrifugation. Matrix effect of real samples were performed on the serum samples.

Results and Discussion

Response characteristics

The equations of the calibrations describing the relation between drug concentration and UV-vis measurements obtained for ciprofloxacin is summarized as:

Ciprofloxacin

Y= 0.0679x+0.0578: (R^2 =0.948): concentration range: 0.1-1 mg/L (Figure 4)

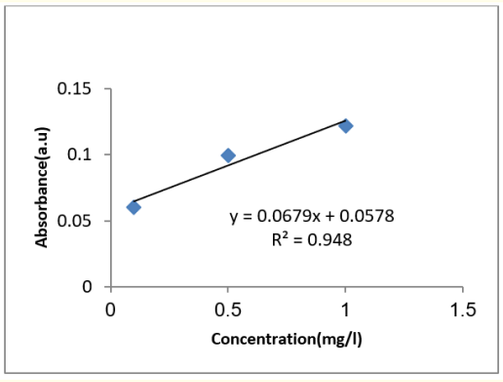

Figure 4: The linear calibration curve in the concentration range (0.1-1 mg/l).

Y= 0.007x+0.1056: (R^2 =0.9554): concentration range: 1-20 mg/L (Figure 5)

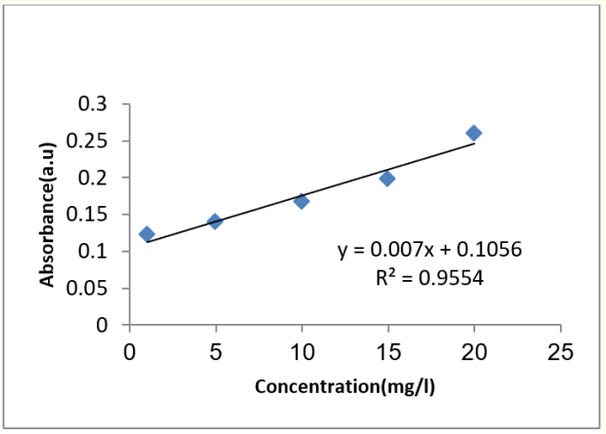

Figure 5: The linear calibration curve in the concentration range (1-20mg/l).

R is the correlation coefficient. The linear concentration ranges were 0.1-20 mg/L for ciprofloxacin based on UV-Vis measurements. The mean percentage recovery ranged from 83 to 104%.

Optimum reaction conditions

The best reaction conditions for the quantitative determination of ciprofloxacin were established via some preliminary experiments. In order to better performance, factors affecting extraction such as amount of nanoparticles, stirring speed, volume of analyte, pH, dispersing solvent, adsorption time, effects of ionic strength and salt, desorption solvent and desorption time were examined by measuring the absorbance of solutions containing a fixed concentration of ciprofloxacin (10 mg/L).

Selecting the optimum solvent for an efficient extraction

To select the suitable solvent, absorbance values caused by the presence of ciprofloxacin measured after extraction with each of different organic solvents. The most suitable solvent for the extraction of ciprofloxacin in the presence of Magnetic Fe_3O_4/SiO_2 nanoparticles was ethanol.

Effect of pH

Substantially, it is anticipated that the maximum efficiency of extraction has occurred when pH of the aqueous solution is in the range of pK_a of the analysts in which analysts remain in a molecular form. As for ciprofloxacin, according to its structure (Figure 1) and the fact that pK_a of carboxylic acid and nitrogen of piperazinyl groups in this drug is 6.09 and 8.74, respectively, different pH values of 2, 3, 5, 7, 9 and 10 were examined. From (Figure 6) it is deduced that the highest absorption for ciprofloxacin was obtained in the neutral range and the best separation is around pH7.

Figure 6: pH effect on the extraction.

Amounts of nanoparticles

The 3,5,8,10 mg of nanoparticle were investigated. According to the figure 5, the best absorbance obtained for 5 mg of the nanoparticle (Figure 7).

Figure 7: Effect of the nanoparticles amount (mg).

Effect of extraction time

Extraction time is an important parameter in the extraction efficiency. The influence of absorption time was examined in the range of the 1–20 min By increasing the time, analyte molecules have a sufficient time to transfer from donor phase to acceptor phase and the absorption factor generally increased. As seen as in (Figure 8) after 5 min, adsorption is reached to a maximum and the extraction efficiency remained constant. Therefore, the extraction time of 5 min was chosen as the optimal time in experiments.

Effect of desorption time

The time required for removing analyte from the sorbent is desorption time. The influence of extraction time was examined in the range of the 5–20 min. Up to the 5 min, a transparent solution had not obtained, so the recording of the absorption through UV-Vis spectrophotometer was impossible. Therefore, the extraction time of 10 min was chosen as the optimal desorption time (Figure 9).

Figure 8: Adsorption time(min) effect on the extraction efficiency.

Figure 9: Desorption time(min) effect on the extraction efficiency.

Effect of stirring rate

Agitation plays a dominant role in the extraction efficiency. Increasing the stirring speed of the sample solution increases the mass transfer from the donor phase to the acceptor phase. The influence of the stirring rate was tested in the range of 200–600 rpm and the rate was optimized. The extraction efficiency of the analyte increased with stirring speed up to 400 and 500 rpm for absorption and desorption experiments, respectively. According to the obtained results depicted in (Figure 10) and (Figure 11), a stirring speed of 400 and 500 rpm was selected as the optimum stirring rate for absorption and desorption stage, respectively.

Figure 10: Stirring rate effect on the adsorption process (rpm).

Figure 11: Stirring rate effect on the adsorption process (rpm).

Effect of desorption solvent type

Several desorption solvents such as ethanol, methanol, nitric acid and acetic acid were examined. According to the obtained results, methanol and ethanol revealed high extraction efficiency and because of dissolving nanoparticles, acetic acid was found to be inappropriate in this case. Therefore, 3 mL ethanol was selected as the suitable solvent (Figure 12).

Figure 12: Effect of desorption solvent type.

Effect of ionic strength

Generally, the salting-out effect improves the extraction efficiency with decreasing the solubility of the analyte molecules in the donor aqueous phase and increasing analyte transfer into extractor phase. In the present work, the effect of different concentration of salt on the extraction efficiency was evaluated by adding NaCl and KCl in the range of 0–5% (w/v).

The experimental results showed a negative effect of salt addition for measurement of ciprofloxacin in the presence of magnetic Fe_3O_4/SiO_2 nanoparticles because by increasing salt concentration, probably viscosity of the solution increases and the diffusion rate, as well as absorption of the analyte on the surface of the nanoparticle, reduces. Therefore, no salt is added to the sample solution (Figure 13 and Figure 14).

Figure 13: Addition of salt effect on the extraction. NaCl(w/v).

Figure 14: Addition of salt effect on the extraction. KCl(w/v).

Effect of Surfactant (Triton X-100)

Sometimes, the presence of non-ionic surfactants reduces the surface tension and improves extraction. Triton X-100 was tested

as non-ionic surfactants in the present work. As shown in (Figure 15), the surfactants had no effect on extraction.

Figure 15: Effect of surfactant, Triton X-100 amount.

Real sample analysis

In order to study the capability of the extraction method in analyzing real samples, the method of standard addition was applied for the analysis of human blood. To measure ciprofloxacin in real samples, different blood serum samples from various men aged in the range of 30-45 were mixed together. Finally, the suitable amount of the mixture was analyzed by this method under the optimized conditions. Only an ultra-trace level of ciprofloxacin was found (under the detection limit). The accuracy of the method was estimated by calculation relative recovery (RR%) carried out with spiking serum samples with a specific volume of aqueous standard solution (5 ml) with concentration range of 0, 0.5, 1, 1.5, 2 (mg/L). The result showed that the relative recoveries of ciprofloxacin (n = 3) ranged from 83-104%.

Conclusions

Solid Phase Extraction (SPE) is the most widely used extraction method for the clean-up, pre-concentration and separation of drug residue from a number of samples. As well the development of new modified adsorbents which were introduced, magnetically modified sorbents were applied to bioseparation and drug analysis.

The proposed method is economically practicable due to the small volume of consuming materials. because of the magnetic nature of the nanoadsorbent, dissembling is quick, easy and takes less time also, sample preparation is simple and does not require applying method. High condensation and good repeatability that reduces relative standard deviations is one of the main advantages of this method.

On the other hand, The UV-Vis method is sensitive, simple and rapid, moreover, it can be used for routine analysis of the investigated drugs in raw materials and pharmaceutical formulations. The statistical parameters and the recovery tests data clearly indicate the reproducibility and accuracy of the method. The proposed modified SPE method seems to provide a simple, and affordable alternative for the routine methods of SPE for drug determination.

Acknowledgements

The authors are thankful to Payame Noor University of Mashhad, Iran, for supporting this research.

Bibliography

1. Z Meng., *et al*. "Residues investigation of fluoroquinolones and sulphonamides and their metabolites in bovine milk by quantification and confirmation using ultra-performance liquid chromatography-tandem mass spectrometry". *Food Chemistry* 174 (2015): 597.

2. Y Pico and V Andreu. "Fluoroquinolones in soil--risks and challenges". *Analytical and Bioanalytical Chemistry* 387 (2007): 1287.

3. M Andersson and MacGowan. "Development of the quinolones". *Journal of Antimicrobial Chemotherapy* 51 (2003): 1.

4. P Sharma., *et al*. "Fluoroquinolone antibacterials: a review on chemistry, microbiology and therapeutic prospects". *Acta Poloniae Pharmaceutica* 66 (2009): 587.

5. B Singh., *et al*. "High performance thin-layer chromatographic selective and stability indicating method for assay of ciprofloxacin in pharmaceuticals". *Der Pharma Chemica* 2 (2010): 178.

6. P Sibinovic., *et al*. "Ruggedness testing of an HPLC method for the determination of ciprofloxacin". *Journal of the Serbian Chemical Society* 7 (2005): 979.

7. B Witte., *et al*. "Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography". *Journal of Chromatography A* 1140 (2007): 126.

8. S Ali., *et al*. Journal of Applied Pharmaceutical Science 1 (2011): 239.

9. M Qureshi., *et al*. Science, Technology and Development, 31 (2012): 69.

10. W Shihn – Sheng., *et al*. "Analysis of ciprofloxacin by a simple high-performance liquid chromatography method". *Journal of Chromatographic Science* 46 (2008): 490.

11. N Kassab., *et al*. Journal of Pharmaceutical Sciences 41 (2005): 507.

12. S Thoppil and P Amin. Journal of Pharmaceutical and Biomedical Analysis 22 (2000): 699.

13. S Joshi. "HPLC separation of antibiotics present in formulated and unformulated samples". *Journal of Pharmaceutical and Biomedical Analysis* 28 (2002): 795.

14. A Faria., *et al*. Journal of the Brazilian Chemical Society 19 (2008): 389.

15. S Zhang and S Wei. Bulletin of the Korean Chemical Society 28 (2007): 543.

16. F Faridbod., *et al*. "Ciprofloxacin Nano-Composite Carbon Paste and PVC Membrane Potentiometric Sensors". *International Journal of Electrochemical Science* 7 (2012): 3693.

17. A Abdala., *et al*. "Differential electrolytic potentiometric titration method for the determination of ciprofloxacin in drug formulations". *Talanta* 61 (2003): 239.

18. C Eboka., *et al*. "Colorimetric determination of the fluoroquinolones". *Journal of Antimicrobial Chemotherapy* 39 (1997): 639.

19. S Pandey., *et al*. "FTIR Spectroscopy: A Tool for Quantitative Analysis of Ciprofloxacin in Tablets". Indian J. Pharm. Sci. 74 (2012): 86.

20. Adegoke., *et al*. International Journal of Pharmaceutical Sciences Review and Research 4 (2010): 1.

21. A El-Brashy,., *et al*. "Spectrophotometric Determination of Some Fluoroquinolone Antibacterials by Ion-pair Complex Formation with Cobalt (II) Tetrathiocyanate". *Journal of the Chinese Chemical Society* 52 (2005): 77.

22. E Cazedey and H Salgado. "Spectrophotometric Determination of Ciprofloxacin Hydrochloride in Ophthalmic Solution". *Advances in Analytical Chemistry* 2 (2012): 74.

23. A Igboasouiui., *et al*. International Journal of innovative research and development 3 (2014): 177.

24. K Rekha., *et al*. International Journal of Advances in Scientific Research 1 (2015): 137.

25. Z Khammas and N Mubdir. Science Journal of Analytical Chemistry 2 (2014): 47.

26. G Ragab and A Amin. "Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of fluoroquinolone antibiotics using ammonium reineckate ion-pair complex formation". *Spectrochim Acta Part A* 60 (2004): 973.

27. P Ramanna., *et al*. International Journal of Pharmaceutical Research and Bio-Science 3 (2014): 50.

28. M Abdel-Hay., *et al*. Journal of the Chinese Chemical Society 55 (2008): 818.

29. J Shah., *et al*. American Journal of Analytical Chemistry 4 (2013): 521.

30. A Abulkibash., *et al*. Talanta, 61 (2003): 239.

31. M Gonzalez., *et al*. Antimicrobial Agents and Chemotherapy 26 (1984): 741.

32. K Sowinski., *et al*. Journal of Clinical Pharmacy and Therapeutics 24 (2004): 381.

33. G Ragab and A Amin. Spectrochim Acta A 60 (2004): 973.

34. M ISSA., *et al*. Journal of the Serbian Chemical Society73 (2008): 569.

35. B Nagaralli., *et al*. "Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations". J. Pharm. Biomed. Anal., 2002, 29, 859.

36. C Lu., *et al*. "Crystalline nanotubes of γ-AlOOH and γ-Al2O3: hydrothermal synthesis, formation mechanism and catalytic performance". *Nanotechnology* 20 (2009): 215604.

37. J Wang., *et al*. "Amino-functionalized Fe(3)O(4)@SiO(2) core-shell magnetic nanomaterial as a novel adsorbent for aqueous heavy metals removal". A*dvances in Colloid and Interface Science* 349 (2010): 293.

38. F Cheng, *et al*. " Characterization of aqueous dispersions of Fe(3)O(4) nanoparticles and their biomedical applications". *Biomaterials* 26 (2005): 729.

Volume 3 Issue 4 April 2019