

Reusable and sustainable graphene oxide/metal–organic framework-74/Fe₃O₄/polytyramine nanocomposite for simultaneous trace level quantification of five fluoroquinolones in egg samples by high performance liquid chromatography

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ABSTRACT

A nanohybrid material termed graphene oxide/metal-organic framework-74/Fe₃O₄/polytyramine (GO/MOF-74/Fe₃O₄/PTy) was fabricated and applied in magnetic dispersive micro-solid phase extraction (MD-μ-SPE) coupled with high performance liquid chromatography (HPLC) for simultaneous determination of fluoroquinolones compounds including, ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sparfloxacin in egg samples. The GO/MOF-74/Fe₃O₄/PTy nanocomposite was fabricated through an in situ synthesis of MOF-74 in the presence of magnetic GO and followed with an oxidative polymerization of tyramine using horseradish peroxidase (HRP) enzyme. The modifier agents improved the merits of the nanoporous sorbent. Extraction protocols based on GO/MOF nanocomposites have various benefits such as, the high stability, the tunable porosity, the fast mass transfer and reasonable enrichment factor. The fabricated material was characterized via energy dispersive x-ray analysis (EDX), the scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR), and the x-ray diffraction (XRD). The calibration curves revealed linearity ($0.9992 \leq r^2 \leq 0.9997$) in the ranges of 1.0–475.0, 0.5–350.0, 0.5–350.0, 0.5–375.0 and 1.5–300.0 ng mL⁻¹ with limit of detections (LODs, S/N=3) of 0.3, 0.1, 0.2, 0.1 and 0.4 ng mL⁻¹ for ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sparfloxacin, respectively. The intra-assay ($\leq 7.7\%$, $n = 9$) and inter-assay ($\leq 7.0\%$, $n = 9$) precisions along with accuracy less than 9.0% showed the reliability of the method.

1. Introduction

Fluoroquinolones (FQs) such as ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sparfloxacin (Fig. 1) have great importance due to

their high antibacterial activity and considerable bioavailability which makes these compounds as efficient drugs not only for treatment of human's diseases but also for prevention and treatment of veterinary illnesses [1]. In recent years, the FQs have been widely used in different infectious diseases due to resistance against these drugs,

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the various hazardous side effects and allergic problems [2]. Based on FQs application in animal husbandry, the impact of FQs has been found in a variety of food samples like milk, the bee products, chicken and eggs. Since eggs and egg yolk products have high protein content and some essential

minerals, they are tremendously utilized in the diet of breastfed children, infants, premature babies and adults. Hence, it is necessary to expand reliable and cost-effective analytical methods to quantify trace amount of FQs residue in egg samples to ensure public health safety in humans [3-5].

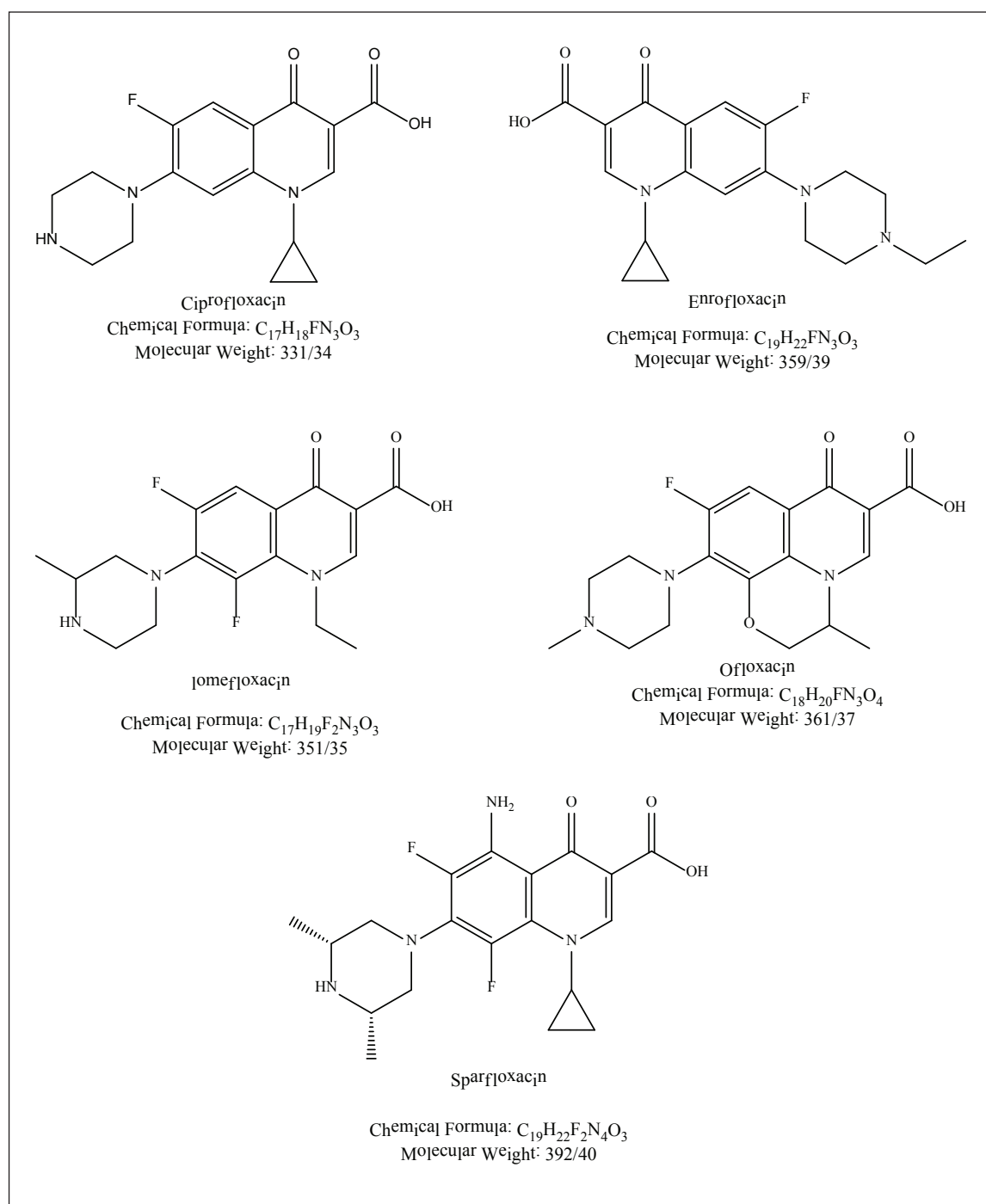


Fig. 1. Structures of target of fluoroquinolones (FQs) such as ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sparfloxacin

Literature survey shows that different analytical protocols have been reported for the determination of FQs which suffer from major drawbacks including high matrix effect, low sensitivity, unacceptable reproducibility, high usage of hazardous reagents and etc. [1, 4-6]. In order to overcome these weaknesses, the development a sustainable sample preparation strategy prior to measurement is essential. Magnetic dispersive micro-solid phase extraction (MD- μ -SPE) as a promising kind of solid-phase extraction (SPE) offers many merits over other traditional sample preparation methods and reveals notable applications in enrichment and isolation of target analyses from complex matrices like environmental, natural, drug and food samples [7-10]. The major advantages of this new kind of extraction method involve significant reduction of toxic reagent usage, removal of time consuming steps like filtration and centrifugation, considerable automation ability and reasonable extraction yields along with a meaningful decrease of interference effects [1]. Researchers have conducted extensive studies over new magnetic sorbents MD- μ -SPE and used in different analytical purposes. However, the developed sorbents show some disadvantages involving the lack of reusability, low surface area, insufficient porosity, low saturation and so on [11]. To deal with these issues, graphene oxide (GO) nanosheet as a novel allotrope of carbon seems superior choice to fabricate a new nanohybrid material for extraction goals. Its high surface area, strong hydrophobic properties, notable mechanical characteristics, outstanding acid and alkaline resistance as well as high chemical and thermal stability, enable it to create increasing π - π interactions [12, 13]. Lately, various materials with individual properties such as silicon-based compounds [14], inorganic nano-materials [15], metal oxides [16, 17], conducting metal polymers [18] and MOFs [19-21] have been introduced as surface modifiers to enhance the merits of the GO. MOFs are classified a new type of highly porous materials which can be synthesized through an interaction between coordinates of metal ions (nodes) and bridging ligands, under appropriate

conditions. MOFs as 3-dimensional structures exhibit various topologies along with individual properties like tunable porosity, high surface area from 1000 to 10400 m²g⁻¹, simple synthesis routes, and adequate resistance. Owing to these possessions, these materials have been applied in different areas like the adsorption phenomena [22], the separation [23-25], the gas storage [26, 27] and the drug delivery [28]. Literature survey shows that some MOFs including Hkust-1 [29], MIL-101 [30], MOF-5 [31], UiO-66 [32], MIL-100 [33] have been applied in SPE as successful sorbents. Among of MOFs crystals, the MOF-74 is resulted from the reaction of divalent metal cations like Mg, Mn, Fe, Co, Ni and Zn with divergent organic ligand called 2,5-dihydroxybenzene-1,4-dicarboxylate (DBDC) [34-36]. In order to enhance the qualities of a carbon based material for extraction purposes, conductive polymers such as polythionine, polyaniline, polythiophene, polytyramine (PTy) seem appreciable alternatives which significantly increase π - π interaction, hydrophobic property, extraction capacity, diffusion rate and reusability [37-43]. Among these polymers, PTy can be synthesized through a simple and inexpensive oxidative polymerization route in the presence of horseradish peroxidase (HRP) enzyme as a catalyst and furthermore existence of alkyl groups in this polymer results the establishment of macropores on the surface of sorbent. The synthesized nanosorbent have been used in a similar way to investigate the pharmacokinetic drugs on human plasma but in this study, as a novel research, we used synthesized sorbent to quantitatively probe the simultaneous, five type of fluoroquinolone in egg sample [44].

In the presented study, the surface of GO nanosheet was modified with MOF-74 to result GO/MOF-74 and furthermore in order to provide a super-magnetic material, precipitation of Fe₃O₄ on the fabricated sorbent was followed. In the last step, the polymerization of tyramine was carried out using HRP enzyme to prepare recyclable GO/MOF-74/Fe₃O₄/PTy nanocomposite as a sustainable sorbent for the MD- μ -SPE process. Ultimately, the extraction protocol was followed with HPLC-

UV for simultaneous extraction and quantitation of five fluoroquinolones including ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sperfloxacin in egg samples and satisfactory precisions along with desirable accuracies were obtained.

2. Experimental

2.1. Chemicals

In this study, the analytical grade of chemicals and reagents were applied. These chemicals involving 2, 5- Dihydroxy triphthalic acid, N, N- dimethylformamide, tyramine, horseradish peroxidase and graphite powder (mesh of 100) were obtained from Merck Company (Darmstadt, Germany). Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), the sodium nitrate (NaNO_3), the potassium permanganate (KMnO_4), the sulfuric acid (H_2SO_4 , 98%), the nickel (II) nitrate hexahydrate [$\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], the sodium hydroxide (NaOH), the hydrochloric acid (HCl 37%), the hydrogen peroxide (H_2O_2 , 30%), the ethanol ($\text{C}_2\text{H}_5\text{OH}$) and triethylamine were obtained from Sigma-Aldrich Company (St. Luise, MO, USA). The standards of fluoroquinolones were provided from Kusum Healthcare (Punjab, India). Ultrapure water (Millipore, Bedford, MA, USA) was used in all experminets. HPLC grades of methanol, acetonitrile, acetone and potassium dihydrogen phosphate were bought from Merck company (Darmstadt, Germany).

2.2. Instrumentation

Energy dispersive x-ray (EDX) spectra and scanning electron microscopy (SEM) images were investigated in detail via a TESCAN-Vega 3 (TESCAN, Czech Republic), machines. All the X-ray diffraction (XRD) spectra were recorded and studied within angular range of $0-80^\circ$, using $\text{K}\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$) created by Cu element on a D8 Advance AXS diffractometer instrument (Bruker, Germany). All the FTIR spectra were recorded on a Perkin Elmer FTIR spectrometer (RXI, Germany).

2.3. Chromatographic analysis

Chromatographic data were obtained using a waters alliance e2695 instrument (Massachusetts, USA) equipped with two pumps for delivering the mobile phase during gradient elution. UV-VIS detector (wavelengths of 275 and 288 nm) and C_{18} reversed phase column ($5 \mu\text{m}$, $250 \times 4.6 \text{ mm id}$, phenomenex Co, Torrance, CA) at 30°C temperature were utilized to complete separation process. A gradient elution containing two types of mobile phases (A: phosphate buffer at pH 3 and B: acetonitrile) was programmed as follows: it was started at 70% A for 12 min, increased to 85% A over 1 min and retained at this value for 6 min and decreased to 70% A for 4 min. The flow rate of pump was regulated at 1 mL min^{-1} in all experiments while the injection volume was set at $20 \mu\text{L}$. The applied mobile phase was filtered through a $0.2 \mu\text{m}$ membrane filter (Millipore, Bedford, MA, USA) for further purification.

2.4. Synthesis

2.4.1. Synthesis of GO/MOF-74

0.25 g graphene oxide (GO was fabricated using hummer's method [45] and 0.23 g of 2, 5-dihydroxyphthalic acid and 1.13 g of $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ were mixed completely and a mixture containing N,N-Dimethylformamide (DMF), ethanol and once ionized water (1:1:1, V/V/V, 100 ml) was added slowly to the above materials. For making a suspension, the obtained solution was sonicated in an ultrasound bath for 10 minutes [46]. The resultant was kept inside an oven for 24 hours at a temperature of 100°C and then it was cooled to 25°C . The upper phase was decanted off and the sedimented phase was washed with methanol for 6 times to remove any impurities.

2.4.2. Synthesis of GO/MOF-74/ Fe_3O_4

For synthesis of GO/MOF-74/ Fe_3O_4 , 0.8 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were mixed and dissolved in 25 mL deionized water. The fabricated GO/MOF-74 was added slowly to the solution under a stream of nitrogen and the pH of the solution was fixed at 10 using ammonia. The

resulting solution was placed into an oven at 100 °C for 24 hours and afterwards it was cooled to 25 °C (room temperature). To remove probable impurities, the upper phase was poured off and residue was washed for 3 times with methanol. The final compound was transferred into an oven with a temperature of 250°C and kept there for 2 hours to achieve a brown powder.

2.4.3. Synthesis of GO/MOF-74/Fe₃O₄/polytyramine

In order to fabricate GO/MOF-74/Fe₃O₄/PTy, an in situ oxidative polymerization was performed on the surface of GO/MOF-74/Fe₃O₄ through an enzymatic cross-linking of poly-tyramine in the presence of HRP enzyme as a catalyst. HRP is considered as a heme-containing oxidoreductase which composes of two broad classes of iron centers including a single heme group [iron (iii) protoporphyrin IX] and two calcium atoms, which catalyzes the oxidation of different organic substrates by hydrogen peroxide. The chemical equation below describes the relevant chemical reaction:

where tyramine as an enzyme substrate, conjugates with hydrogen peroxide and thus catalyzed by HRP. Synthesis route was as follows: 200 mg GO/MOF-74/Fe₃O₄, 160 mg tyramine, 1 mg HRP, 8 mL acetone and 4 mL phosphate buffer (0.1 M, pH 7) were completely mixed together. Then, 240 µL hydrogen peroxide was used to proceed the reaction at a temperature of 30°C. The obtained solution was filtered and placed into an oven and kept there until dryness.

2.5. Preparation of standard solutions and quality control samples of FQs

In order to prepare stock solutions, required amount of each FQs including ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sperfloxacin was independently dissolved in methanol to result a concentration of 10.0 mg L⁻¹. To prevent the decomposition of FQs, the stock solutions of these drugs were prepared every week and stored in the dark place at 4 °C. To obtain working standard solutions of FQs, stock solutions were diluted step

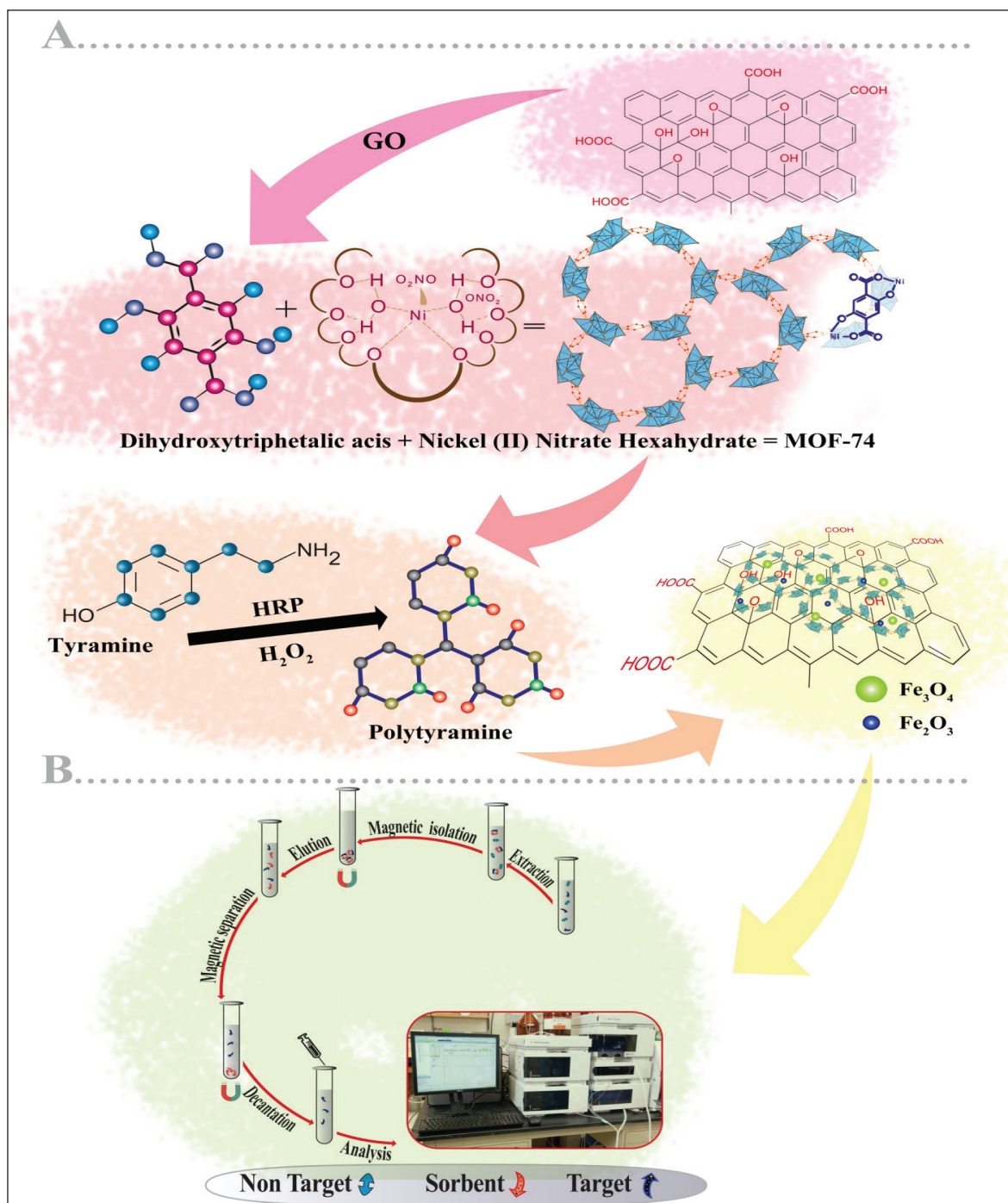
wise. Egg samples were spiked with various levels of the working standard solutions for plotting calibration curve and further measuring. To demonstrate the accuracy and reproducibility of the presented method, different quality control samples of target FQs at concentration levels of 10.0, 150.0 and 350.0 ng mL⁻¹ were prepared.

2.6. Preparation of egg samples

Two kinds of egg samples (subject) were collected and prepared before using by the proposed method. The egg samples 1 from healthy hens without feeding any drugs for evaluation of inter-day/ intra-day precisions and accuracies were used. The egg samples 2 from hens which had been fed a certain amount of five FQs once a day for 7 day for conducting recovery experiments and evaluating the reliability of the method were selected. In order to prepare egg samples, 5 g of eggs was added to centrifuge tubes and after that 10 mL methanol was added to them and centrifuged at 5000 rpm for 10 min. The upper phase was decanted to the new tubes and evaporated to dryness under a stream of nitrogen. Finally, 5 mL deionized water was added to each tube and subjected to the presented extraction protocol.

2.7. The procedure of MD-µ-SPE-HPLC-UV

The steps of MD-µ-SPE-HPLC-UV procedure for isolation, enrichment and quantitation of FQs are shown in [schema 1](#). Firstly, 5.0 mL of the prepared egg sample was placed into a centrifuge tube and then 10.0 mg of GO/MOF-74/Fe₃O₄/PTy was added to the tube containing the real sample. After that, ultrasonic irradiation was utilized for 10 min, to disperse the supermagnetic nanoporous sorbent into the solution and isolate the analytes of interest. The tube containing the sample was exposed to a powerful magnet Nd-Fe-B with a magnitude of 0.8 tesla to collect the particles of nanosorbent at the bottom of the vessel. In the next step, the aqueous media was discarded and the remaining extractor was washed with 2.5 mL acetonitrile through the applying ultrasonic irradiation for 2 min. Then, the sample containing desorbed FQs was exposed to



Schema 1. A: Schematic diagram of the synthesis routes of GO/MOF-74/Fe₃O₄/PTy. B: different steps of MD- μ -SPE-HPLC-UV method in extraction, enrichment and isolation of FQs

the magnet again to collect solution. The collected solution was evaporated under a stream of nitrogen to dryness and the resultant residue was dissolved in 100.0 μL of the optimized mobile phase of HPLC. Finally, 20.0 μL of the obtained sample was injected into HPLC for analyzing FQs.

3. Results and Discussion

3.1. Characterizations

The illustrates of the FTIR spectrum of GO/MOF-74/Fe₃O₄/PTy, recorded in the range of 400–4000 cm^{-1} (Fig.2) The FTIR spectrum of the synthesized nanocomposite sorbent indicates all the components of this structure evidently. As clearly exhibited in the spectrum, the broad peak at 3200–3648

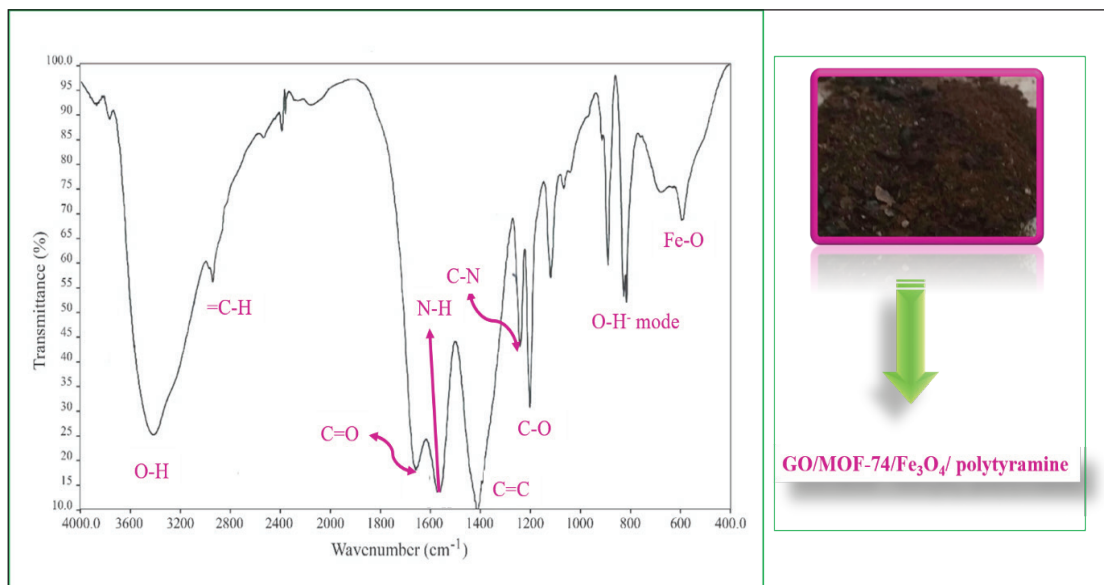


Fig. 2. The FT-IR spectrum of GO/MOF-74/Fe₃O₄/PTy nonporous composite

cm⁻¹ assigns to hydroxyl groups with stretching vibrations in the structure of GO. Oxygen-containing functional groups in the composition of this sorbent include epoxy C-O and C=O stretching vibrations with absorption peaks in the range of 1100-1200 cm⁻¹ and 1650-1680 cm⁻¹, respectively. The absorption peaks corresponding with aromatic absorption bonds with C=C stretching vibrations are located in the range of 1410-1450 cm⁻¹. Due to the adjusted attachment of magnetic Fe₃O₄ on the surface of GO, a peak is observed at 500-580 cm⁻¹, which attributes to the stretching vibrations of Fe-O. μ -hydroxo groups can also be identified in the corner-sharing hexagonal units of MOF-74 with two sharp peaks at 850-950 cm⁻¹ [48]. Other peaks placed in the range of 1210-1250 cm⁻¹ are related to N-H and C-N vibrations, respectively.

In the XRD spectrum (Fig. 3), the peaks associated with GO, GO/MOF-74/Fe₃O₄, and GO/MOF-74/Fe₃O₄/PTy are meticulously compared with each other. The XRD pattern with diffraction peak assigned to (101) at $2\theta=11.281^\circ$, which was shown in Figure 3a, confirms the GO structure. As Figure 3b and 3c, the MOF structure is clearly exhibited in the XRD pattern that confirms its unique crystal structure according to the x-ray reflection indexed to (110) to (300) and diffraction peaks at $2\theta=7^\circ$ and 12° . Also, Fe₃O₄ can be found in the structure

of the nanocomposite sorbent due to the following data: the corresponding x-ray diffraction peaks assigned to (220), (311), (400) at $2\theta=30.3^\circ$, 43.3° , 57.3° , and 62.9° can be seen in the XRD patterns of Figure 3b and 3c, endorsing the presence of Fe₃O₄. Since Fe₂O₃ is also integrated into the fabricated nano-hybrid sorbent, other diffraction peaks at 33.2° , 40.8° , and 35.6° related to (104), (110), (113) plates can be identified, manifesting the presence of Fe₂O₃ in the sorbent as well as Fe₃O₄. Moreover, the grain size and morphology and of nanoporous composite was evaluated by SEM images of GO (d), GO/MOF-74/Fe₃O₄ (e) and GO/MOF-74/Fe₃O₄/PTy (f) in Figure 3. As illustrated in Figure 3d, GO nanosheets were stacked in condensed layers within the lamellar morphological components. Moreover, it shows that wrinkles and folds were observed in robust agglomeration of graphene sheets, which consists of multiple agglomerated layers of graphene, with a strong tendency to stack due to the high surface energy caused by strong interactions of surface groups on the graphene layer. Figure 3e exhibits the crystal growth mechanism and evolution of MOF-74 and Fe₃O₄ particles over the GO nanosheet that resulted in development of sphere-like morphologies, appropriately distributed over GO surface. In Figure 3f, SEM image of GO/MOF-

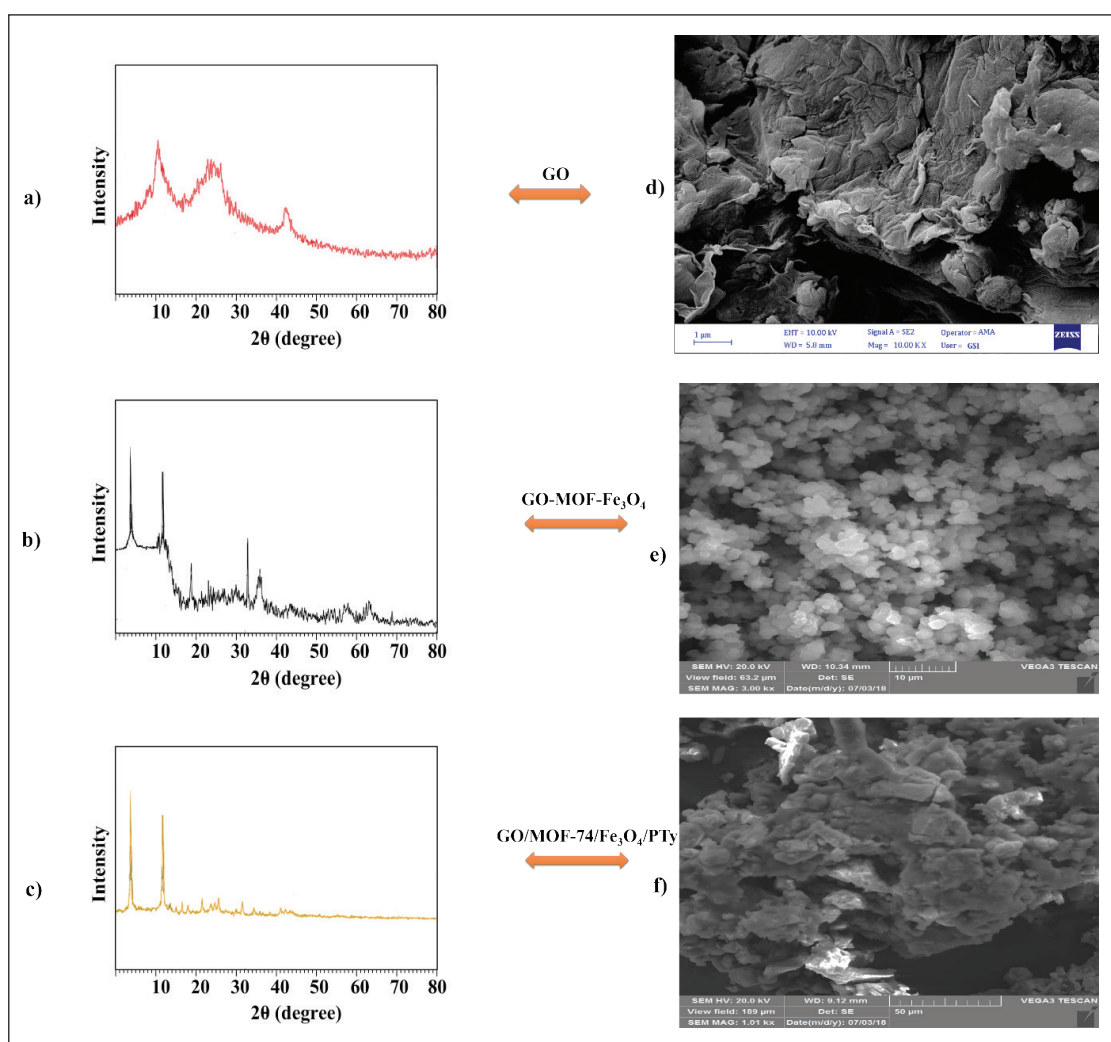


Fig. 3. XRD patterns of GO (a), GO/MOF-74/Fe₃O₄ (b) and GO/MOF-74/Fe₃O₄/PTy (c); SEM images of GO (d), and GO/MOF-74/Fe₃O₄ (e) and GO/MOF-74/Fe₃O₄/PTy (f)

74/Fe₃O₄/ PTy obviously shows the modification process of GO with the layers of target polymer. As it can be seen, PTy layers have grown on the surface of GO and formed significant coating sheets, demonstrating prosperous synthesis of the final nanoscale extractor.

When crystalline MOF-74 particles in Figure 4a are evenly dispersed on the surface of GO sheets, MOF-74 would adhere to GO (Fig. 4b). SEM image of MOF-74 seems to be very similar when compared to GO/MOF-74 image in Figure 4a and 4b. Hence, by referring to EDX spectra of MOF-74 and GO/MOF-74, it could be possible to determine the successful immobilization of MOF-74 on the surface of GO (Fig. 5). The

current data exhibit the homogeneous distribution of the contents of MOF-74 such as Ni, C and O elements in both groups, confirming the effective attachment of MOF-74 blocks with GO layers. For more evidences, an increase in mass percent of carbon material from 31.2 to 49.2% proves the presence of GO in GO/MOF-74 composite, which firmly demonstrates well dispersion of target MOF on the GO sheets.

In Figure 5, the EDX spectrum of GO confirms the existence of C and O elements, with weight percent of 77.1% and 18.5%, respectively. Since MOF-74 is obtained from the reaction between of nickel cation and ligand 2,5-dihydroxybenzene-1,4-dicarboxylate, Ni, C, and O appear at 39.8%,

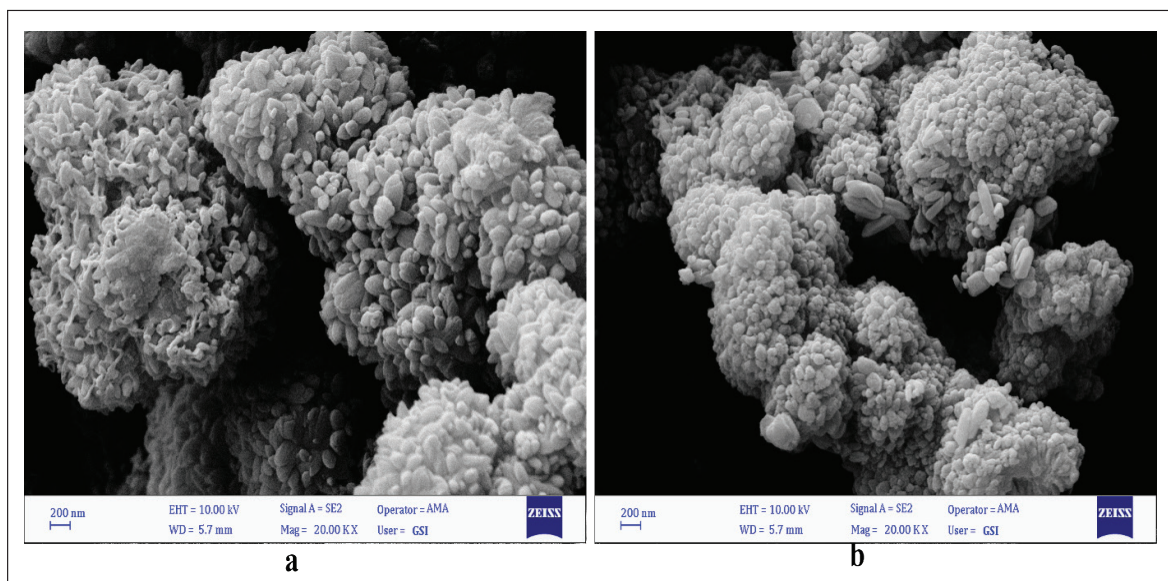


Fig. 4. SEM images of MOF-74(a), GO/MOF-74 (b)

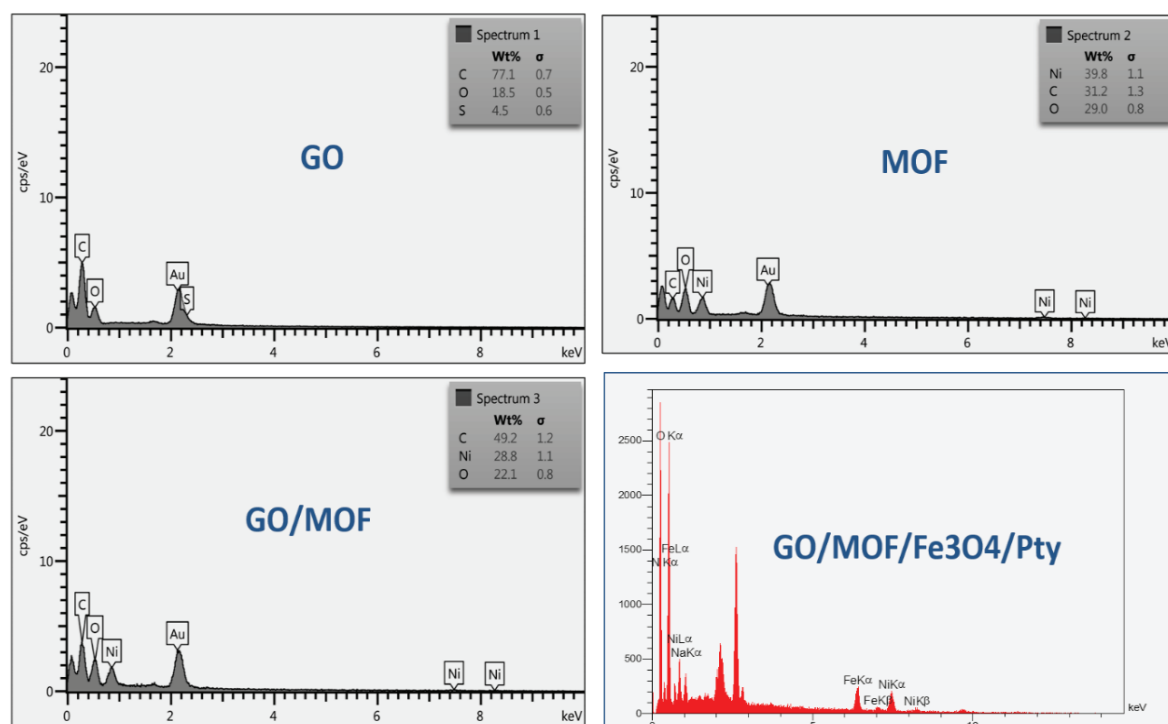


Fig. 5. EDX spectrum of GO, MOF-74, GO/MOF-74 and GO/MOF-74/Fe₃O₄/Pty

31.2%, and 29%, respectively. When MOF is bonded to GO, the carbon content increased from 31.2% to 49.2%, designating the proper adjustment of MOF-74 on the GO surface. In the EDX spectrum of GO/MOF-74/ Fe₃O₄/PTy, Fe and N, associated with Fe₃O₄ and PTy, respectively, can be recognized in addition to previous components.

3.2. Influence of nanosorbent dosage

In the enrichment protocols based on nanoporous sorbents, the amount of extractor is an important factor which effects both reproducibility and sensitivity features [49]. To achieve the best performance of the extraction method for analysis of FQs, various amounts of the fabricated

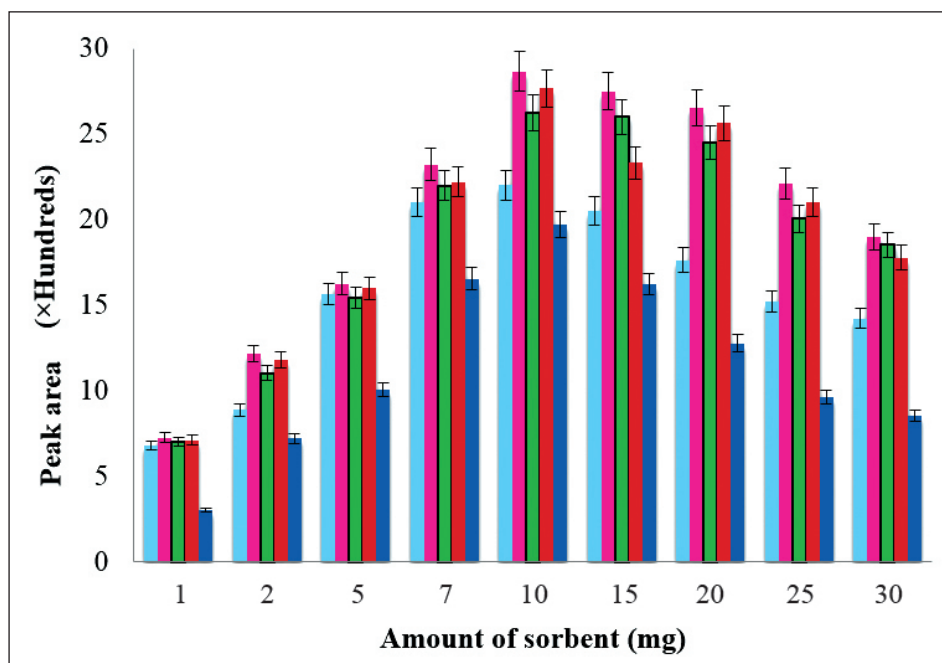


Fig. 6. Influence of GO/MOF-74/Fe₃O₄/PTy composite amount, other conditions: concentration of each FQs 20.0 ng mL⁻¹; pH 5.0; extraction time 10 min; eluting solvent acetonitrile; desorption time 2 min.

nanocomposite within the range of 1.0-30.0 mg were investigated in detail. As Figure 6 shows, there is a significant and direct relationship between peak area of FOs and amounts of nanosorbent from 1.0 to 10.0 mg. GO/MOF-74/Fe₃O₄/PTy has high surface area-to-volume ratio resulting the maximum analytical sensitivity is attainable at a relatively low amount of sorbent (10.0 mg), which can be considered as a prominent advantage of the new designed extractor. But after the value of 10.0 mg, a decrease in signal was observed, which was due to this fact that at higher amounts of sorbent, the separation of analytes from the extractor using the magnet could not be performed effectively and a certain amount of FQs remains in sample. Hence, 10.0 mg of GO/MOF-74/Fe₃O₄/PTy was operative enough to obtain a compromise between analytical sensitivity and repeatability of data, so this value was utilized for the rest of the work.

3.3. Influence of pH

Owing to the presence of carboxyl and amino groups in FQ structures, FQs exist as ionized or neutral forms depending on the pH of the aqueous media, while the reported pK_a values for these

compounds are as follow: (5-5.5) for pK_{a1}, (6.2-6.4) for pK_{a2}, and (8.9-9) for pK_{a3}[5]. pH of the sample media plays a meaningful role on the type of interactions between sorbent and FQs controlling the adsorption phenomena of analytes and the subsequent extraction yields. The impact of pH solution on the determination of FQs was evaluated in the range of 1.0-12.0 by applying 0.01 M HCl and NaOH. As it can be revealed in Figure 7, the best quantification condition was obtained at pH 5.0, which the following explanations exhibit the probable reason: according to the pK_a values of all FQs, at pH 5 the neutral forms (uncharged forms) of drugs are dominant and due to the hydrophobic property of GO/MOF-74/Fe₃O₄/PTy, the hydrophobic-hydrophobic interactions between analytes and sorbent become prevalent at this pH resulting higher recovery values. Hence, pH 5.0 was chosen as the optimum in all enrichment steps, in order to achieve the best performance of the method in the trace monitoring of FQs.

3.4. Influence of ultrasonic irradiation and extraction time

It is well-known that application of ultrasonic

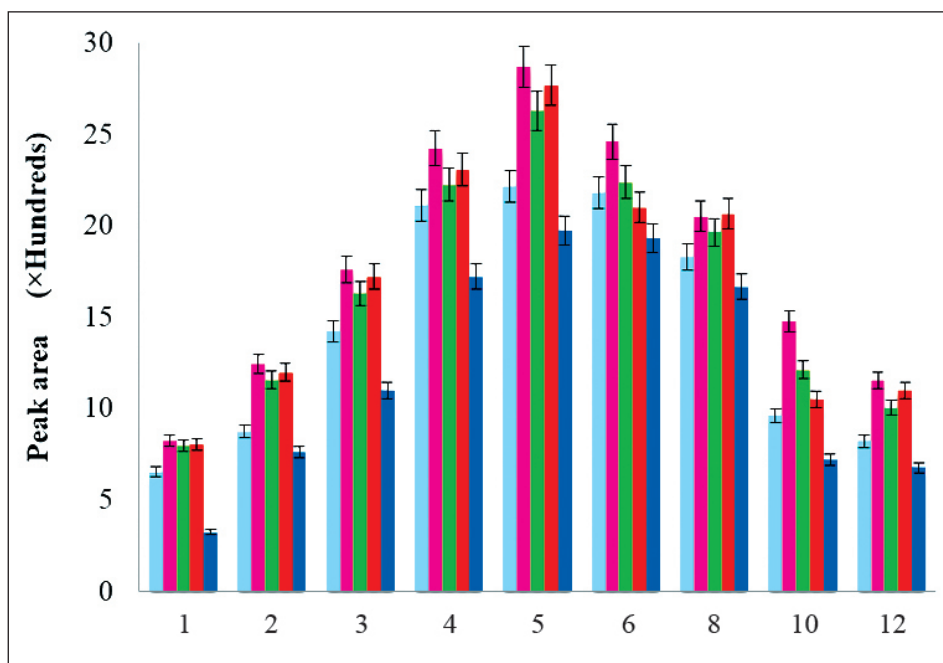


Fig. 7. Influence of sample pH, other conditions: concentration of each FQs 20.0 ng mL⁻¹; sorbent amount 10.0 mg; extraction time 5 min; eluting solvent acetonitrile; desorption time 2 min.

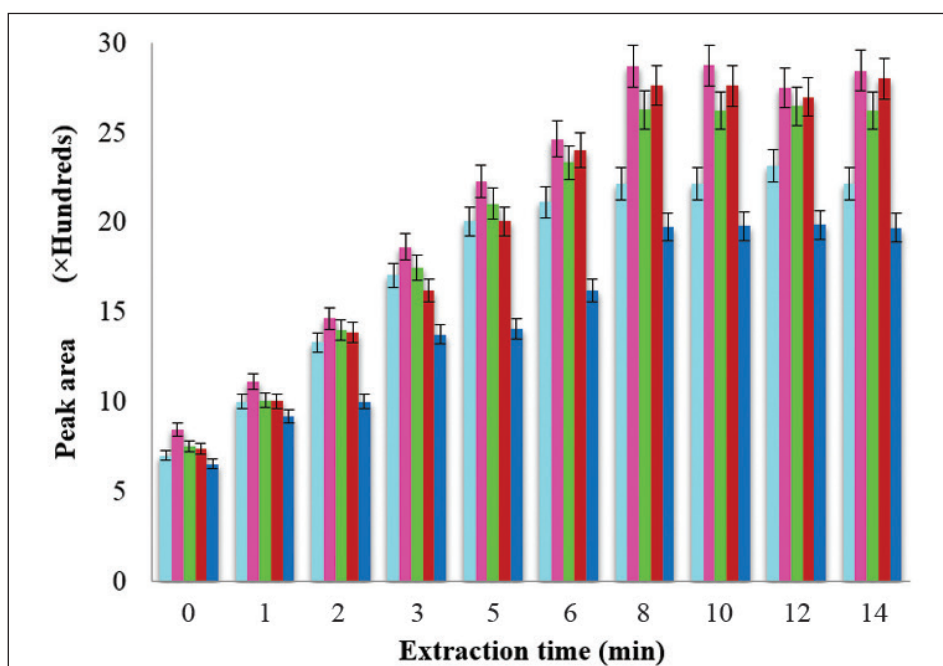


Fig. 8. Influence of extraction time, other conditions: concentration of each FQs 20.0 ng mL⁻¹; sorbent amount 10.0 mg; pH 5.0; eluting solvent acetonitrile; desorption time 2 min.

irradiation has reasonable potential for dispersing the sorbent into the whole sample while the time of irradiation either plays a significant role on a successful extraction process. The time of irradiation is considered as the extraction time which a desirable value causes better mass transfer

and more sensitive signals [50]. The influence of extraction time on analytical signals was examined from 0 to 14 min and the obtained data are shown in Figure 8. Stable and sensitive results were obtained at 10 min revealing a relatively rapid isolation of drugs have been happened, which is due to the high

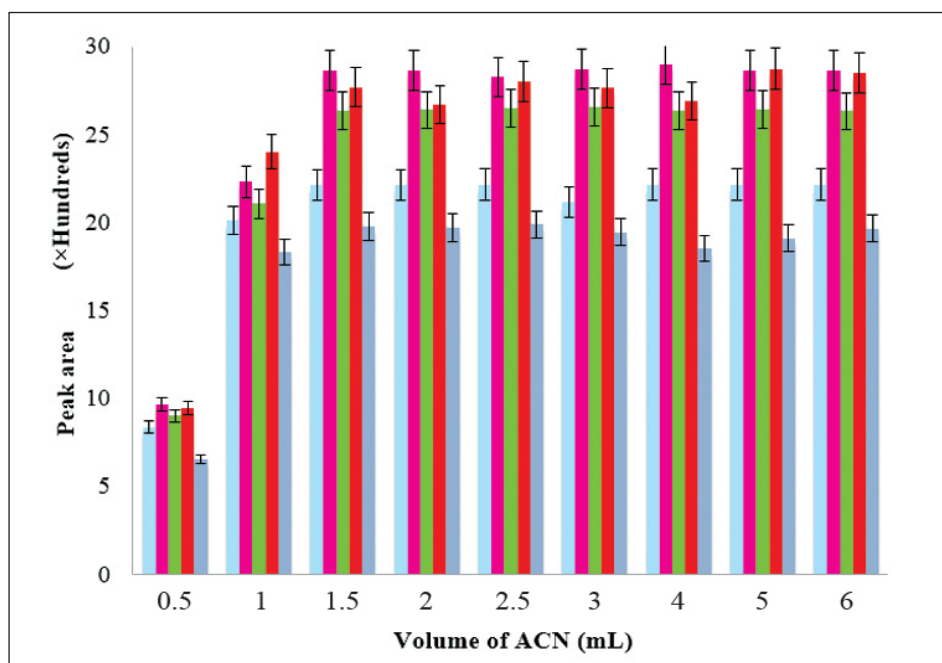


Fig. 9. Influence of acetonitrile volume, other condition: concentration of each FQs 20.0 ng mL⁻¹; sorbent amount 10.0 mg; pH 5.0; extraction time 10 min; desorption time 2 min.

porosity of the sorbent. After 10.0 min the analytical signals slowly decrease owing to this fact that at higher time values, FQs are separated from the sorbent and re-entered to the solution. According to these criteria, 10.0 min was good enough to cover all necessities associated to quantification features and this value was selected in all experiments.

3.5. Desorption condition

To evaluate the best desorption condition, different kinds of organic solvents such as methanol, acetonitrile and acetone as eluting agents with different volumes were tested. The volume and kind of eluting agents significantly affect the enrichment and isolation of target drugs so it is essential to carefully evaluate these parameters in detail. Acetonitrile exhibited individual and more practical desorbing ability in comparison with other solvents. After selecting the kind of solvent, its volume should be taken to the account, hence various volume of acetonitrile from 0.5 to 6.0 mL were subjected to the extraction protocol and as it can be seen in Figure 9, 2.5 mL of this solvent was adequate to deal with all necessary issues and provide reproducible and sensitive data.

3.6. The influence of salt concentration

In analytical chemistry there is a well-known phenomenon termed salting out effect which is based on non-electrolyte-electrolyte interactions. Non-electrolytes are less soluble in water at high values of salt and as a result the adsorption process could be prevalent. Thus, in this study different samples containing NaCl from concentration levels from 0 to 10% w/v were studied to evaluate the salting out effect on the extraction of FQs. It was expected by adding salt, the solubility of analytes in aqueous phase decreases due to an increase in polarity of the aqueous medium, and thus, the extraction performance improves [51, 52] But as it is clear in Figure 10, the analytical sensitivities have been missed by increasing the salt level. The latter happening can be explained as follows: at higher concentrations of NaCl, the sample become more viscous and make the mass transfer of FQs so difficult that results a meaningful decline in analytical signals. Finally, in order to obtain better condition, no electrolyte was used in all evaluations.

3.7. Evaluation of Reusability

To evaluate the reusability of GO/MOF-74/Fe₃O₄/

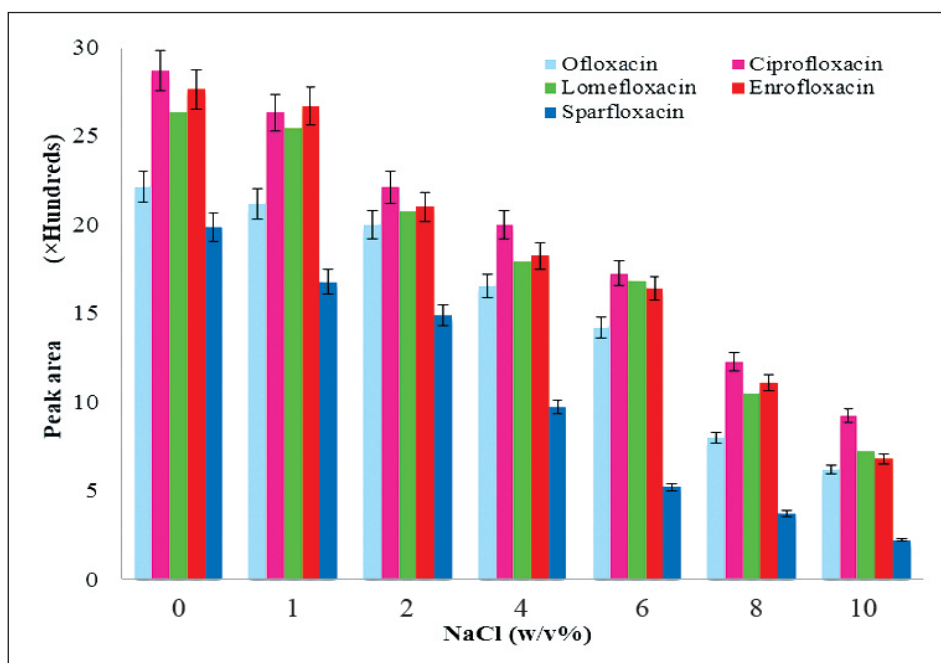


Fig. 10. Influence of ionic strength, other conditions: concentration of each FQs 20.0 ng mL⁻¹; sorbent amount 10.0 mg; pH 5.0; extraction time 10 min; eluting solvent acetonitrile; desorption time 2 min.

PTy, the number of adsorption-desorption cycles must be examined. Reusability is considered an essential characteristic of nanoscale sorbents, in order to reduce the cost of analyses and provide reliable data. To study this capability of magnetic sorbent, after extraction process it was washed with 1.5 mL deionized water and 1.5 mL acetonitrile during the application of ultrasonic for 6 min. Then, the magnetic nanosorbent was allowed to be dried at room temperature and reused for other subsequent extractions. It was found that after 15 adsorption-desorption cycles, the recovery values decreased around 13%.

3.8. Analytical figures of merit

Analytical aspects of the presented MD-μ-SPE-HPLC-UV were evaluated and main features are as follow: calibration curves revealed satisfactory linearity ($0.992 \leq r^2 \leq 0.997$) in the range of 1.0–475.0, 0.5–350.0, 0.5–350.0, 0.5–375.0 and 1.5–300.0 ng mL⁻¹ with limit of detections (LODs, S/N=3) of 0.3, 0.1, 0.2, 0.1 and 0.4 ng mL⁻¹ and limit of quantifications (LOQs, S/N=10) of 1.0, 0.5, 0.5, 0.5 and 1.5 ng mL⁻¹ for ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and sperfloxacin,

respectively. The mentioned analytical figures of merit along with calibration curve equation, extraction recovery (ER) and enrichment factor are summarized in Table 1. EF value of each FQ was defined as the ratio of the calibration curve slope before and after extraction method. ER values were calculated using the equation (I):

$$ER\% = EF \times (V_{\text{Final volume}} / V_{\text{Initial volume}}) \times 100 \quad (\text{Eq. I})$$

Figure 11 shows the HPLC chromatograms for blank sample and egg sample with different spiked level of FQs. The obtained chromatogram exhibits other contaminants existing in the egg samples have no notable effect on the recording of data and subsequent measurements.

3.9. Evaluation of precision and accuracy

Intra-day (within one day) and inter-day (within three days) precisions along with related accuracies were studied and estimated through the analyses of various quality control (QC) samples in the concentration levels of 10, 150 and 350 ng mL⁻¹. Each QC sample was obtained from spiking the drug under study into

Table 1. Various analytical figures of merit of MD- μ -SPE-HPLC-UV.

Analyte	LDR (ng mL ⁻¹)	Linear equation	r^2	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)	EF	ER% ($n=3$)
Ofloxacin	1.0-475.0	$Y=106X+93$	0.992	0.3	1.0	43.6	87.2
Ciprofloxacin	0.5-350.0	$Y=137X+128$	0.996	0.1	0.5	45.0	90.0
Lomefloxacin	0.5-350.0	$Y=126X+114$	0.995	0.2	0.5	43.4	86.8
Enrofloxacin	0.5-375.0	$Y=128X+205$	0.997	0.1	0.5	45.6	91.3
Sparfloxacin	1.5-300.0	$Y=95X+89$	0.995	0.4	1.5	44.2	88.5

LDR: Linear dynamic range; r^2 : Correlation coefficient; LOD: Limit of detection; LOQ: Limit of quantification; EF: Enrichment factor; ER: Extraction recovery

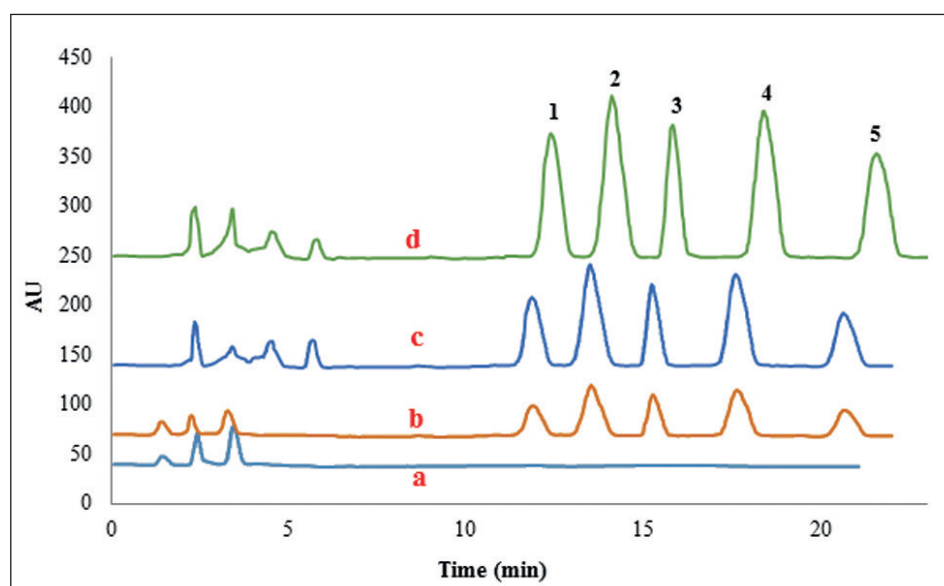


Fig. 11. Various HPLC-UV chromatograms of ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin, sparfloxacin after enrichment protocol: egg sample as the blank after applying MD- μ -SPE (a); Spiked egg samples after applying MD- μ -SPE with concentration level of each FQs at (b) 25.0 ng mL⁻¹, (c) 50.0 ng mL⁻¹, and (d) 150.0 ng mL⁻¹. (1) ofloxacin, (2) ciprofloxacin, (3) lomefloxacin, (4) enrofloxacin, (5) sparfloxacin.

Table 2. Intra-day and inter-day precisions along with corresponding accuracies for trace measurement of ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin, sparfloxacin in spiked egg samples.

Drug	Conc. (ng mL ⁻¹)	Intra-day, <i>n</i> = 9			Inter-day, <i>n</i> = 9		
		Found (ng mL ⁻¹)	RSD (%)	Accuracy (%)	Found (ng mL ⁻¹)	RSD (%)	Accuracy (%)
Ofloxacin	10.0	10.6 ± 0.4	3.8	6.0	9.3 ± 0.5	5.4	-7.0
	150.0	143.9 ± 7.1	4.9	-4.1	141.0 ± 8.0	5.7	-6.0
	350.0	329.5 ± 16.8	5.1	-5.9	371.8 ± 15.6	4.2	6.0
Ciprofloxacin	10.0	9.6 ± 0.4	4.2	-4.0	10.5 ± 0.8	7.6	5.0
	150.0	159.5 ± 8.6	5.4	6.3	162.0 ± 9.0	5.5	8.0
	350.0	362.0 ± 14.4	4.0	3.4	379.3 ± 22.0	5.8	8.4
Lomefloxacin	10.0	10.3 ± 0.6	5.8	3.0	9.4 ± 0.7	7.4	-6.0
	150.0	140.6 ± 5.7	4.0	-6.3	138.5 ± 9.1	6.6	-7.6
	350.0	322.5 ± 15.3	4.7	-7.9	322.4 ± 17.3	5.4	-7.8
Enrofloxacin	10.0	10.6 ± 0.6	5.7	6.0	9.1 ± 0.7	7.7	-9.0
	150.0	155.5 ± 8.2	5.3	3.7	137.0 ± 7.9	5.8	-8.6
	350.0	324.1 ± 22.7	7.0	-7.4	370.4 ± 26.6	7.1	5.8
Sparfloxacin	10.0	10.4 ± 0.5	4.8	4.0	9.3 ± 0.8	8.6	-7.0
	150.0	156.4 ± 7.7	4.9	4.3	140.3 ± 8.3	5.7	-6.5
	350.0	325.9 ± 18.1	5.5	-6.9	321.0 ± 20.4	6.3	-8.3

RSD (%) values are determined as $100 \times \text{SD}/\text{mean}$; Accuracy (%) values were defined as $(\text{mean level found} - \text{known level})/(\text{known level})$; Three independent analyses were obtained for every concentration of target FQs.

the egg sample, in order to examine the developed method in the analysis of real matrix. The results of the recent experiments are completely shown in Table 2. As it can be seen, reasonable intra-assay ($\leq 7.7\%$, $n = 9$), inter-assay ($\leq 7.0\%$, $n = 9$) as well as accuracy values ($< 9.0\%$) were obtained which all demonstrate the reliability of the method for simultaneous trace screening of FQs in real samples.

3.10. Application of the method to egg sample analysis

To check the validity of the MD- μ -SPE-HPLC-UV for simultaneous trace monitoring of FQs in complicated

matrices, it was applied for analyzing target drugs in egg samples. The egg samples from hens which had been fed the drug per day with 7 days were subjected the extraction protocols and the obtained results are summarized in Table 3. Furthermore, egg samples were spiked with different amounts of FQs and after analyzing the samples, the recovery values in three independent measurements were calculated. The recovery values for all FQs varied from 91.0 to 106.8% and relative standard deviations (RSDs%) were in the range of 3.3-7.2% which demonstrate the validity of the method in analysis of complicated real matrices like egg samples.

Table 3. Simultaneous monitoring of ofloxacin, ciprofloxacin, lomefloxacin, enrofloxacin, sparfloxacin in egg samples by the developed method.

Analytes	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	RSD (%; n=3)	Recovery (%)
Ofloxacin	-	5.8	5.3	-
	5	10.1	4.9	93.5
	25	28.0	6.0	91.0
	50	59.6	5.9	106.8
Ciprofloxacin	-	3.7	4.8	-
	2	5.3	6.0	93.0
	25	26.6	5.5	93.9
	50	50.7	5.4	94.4
Lomefloxacin	-	3.1	5.1	-
	2	4.7	7.2	92.2
	25	29.5	4.3	105.0
	50	49.7	4.0	93.6
Enrofloxacin	-	14.5	3.3	-
	2	15.2	4.9	92.1
	25	37.0	4.4	93.7
	50	59.7	5.0	92.6
Sparfloxacin	0	8.2	4.8	-
	5	12.3	5.2	93.2
	25	31.2	6.8	94.0
	50	53.8	6.0	92.4

Three independent measurements were carried out for each concentration level and mean values were calculated.

3.11. Comparison with other methods

To highlight the robustness of the method, major analytical figures of merit including RSD, LOD, LOQ, correlation coefficient (r^2) along with some extraction features were compared with previously reported methods in literature. The results of the current investigation are summarized in Table 4. As it can be seen, the developed protocol exhibits notable improvements in approximately all analytical features besides a comparable extraction time over other reported procedure. Furthermore, the MD- μ -SPE-HPLC-UV reveal some worthy advantages like a reduction in the usage of toxic solvent in comparison with conventional SPE methods, opportune magnetic separation with the no need of individual device, easy-to-recycle the magnetic nanosorbent that can be used more than 14 times along with the possibility of trace simultaneous screening of target drugs with the least interferences.

4. Conclusions

A four-part magnetic nanoporous GO/MOF-74/Fe₃O₄/PTy was effectively fabricated and employed as a capable sorbent for MD- μ -SPE. Surface immobilization of GO with modifier agents including MOF-74, PTy and iron oxide significantly improved the merits of the hybrid materials and provided notable advantages like improvement of aromatic-aromatic interactions, high mass transfer, desired porosity, worthy reusability, easy-to-recycle the magnetic nanosorbent and reasonable recovery values. The results exhibited that MD- μ -SPE in combination with HPLC-UV is a valid protocol of enrichment and simultaneous trace level quantification of fluoroquinolones in real media like egg samples. The satisfactory sensitivity and reproducibility along with acceptable accuracy without considerable interferences from contaminants in egg matrices demonstrated the reliability of the method for trace screening purposes, and according to these criteria it possesses

Table 4. Comparison of MD- μ -SPE-HPLC-UV with other reported methods in literature for quantitation of different fluoroquinolones in eggs.

Extraction Method	Extraction Phase	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	r^2	Extraction time(min)	RSD (%)	Detection system	Ref.
MSPE	C ₁₈	0.5-5	0.7-17	0.9990-7	12	<5	HPLC-UV	[11]
SPME	C ₁₈	*3-10	*10-30	0.986-7	10	NR	**LC	[46]
DSPE	C ₁₈	^a 0.1-2.6	^a 0.4-8.6	0.9996-9	10	1-7	HPLC	[47]
SPE	^b ENVI-18 disk	0.7, 2	2, 6	0.9994-7	5	<10	HPLC	[6]
SFE	C ₁₈	<10	NR	>0.995	50	NR	HPLC-F	[53]
PLE	C ₈ -silica	0.4-33.5	0.2-19.8	NR	>54	<23	HPLC-F	[54]
MSPE	^c PA	^d 0.4-1.4	^d 1.1-4.5	0.9974-1	2	3.6-17.6	HPLC	[55]
MD- μ -SPE	GO/MOF/Fe ₃ O ₄ /PTy	0.1-0.4	0.5-1.5	0.992-7	10	3.3-7.2	HPLC-UV	This work

* $\mu\text{g/kg}$, ** LC-FLC-TMS, ^a $\mu\text{g/g}$, ^b polystyrene-divinylbenzene copolymer disk, ^c PA:One-dimensional polyanilines, ^d ng/g.

MSPE: magnetic solid phase extraction

SPME: solid-phase microextraction

DSPE: dispersive solid phase extraction

PLE: pressurized liquid extraction

NR: not reported

RP-HPLC/UV: Reversed phase high performance liquid chromatography

RP-HPLC-F: reversed phase high-performance liquid chromatography with fluorescence detection

HPLC-UV: high performance liquid chromatography with UV detection

DMIP: dummy molecularly imprinted polymers

appreciable potential to be employed in the other applications related to the food aspects.

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