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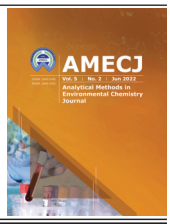

Application of pipette-tip solid-phase extraction technique for fast determination of levofloxacin from wastewater sample using cobalt metal-organic framework

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ABSTRACT

In this research, a miniaturized solid-phase extraction method based on pipette tip solid-phase extraction (PT-SPE) was employed for the determination of levofloxacin. Cobalt metal-organic framework (CoMOF) was used as an adsorbent. Levofloxacin was determined using high-performance liquid chromatography and UV detection (HPLC-UV). Important parameters that influence the extraction efficiency (*i.e.* pH, amount of adsorbent, extraction time, volumes of sample, and eluting solvent) were tested and optimized. Results indicated that the proposed method was validated over the range of 0.70 - 150.0 µg L^{-1}. The relative standard deviation (RSD%) was below 2.75% for the levofloxacin. The limit of detection (LOD) of this method is 0.041 µg L^{-1}. The preconcentration factor (PF) was obtained at 200 and the analysis time was around 10 min that confirming the reliability and accuracy of this method for extraction of levofloxacin. The PT-SPE procedure based on CoMOF adsorbent was efficiently extracted for levofloxacin more than 95%. In a static system, the adsorption capacity of CoMOF adsorbent for levofloxacin was obtained at 156.7 mg g^{-1} (n=10). The validation of results was successfully obtained for levofloxacin values based on the spiking real samples before determination by the HPLC technique.

1. Introduction

Antibiotics are among the drugs that are widely used in medicine and veterinary medicine and through various routes such as agricultural runoff, direct discharge from municipal wastewater treatment plants, human waste, direct disposal of medical waste, veterinary, and industry[1, 2]. As a result, their presence has been observed in local streams and around the world, especially in streams that directly receive treated wastewater[3]. Large amounts of these compound residues remain in agricultural soils after being applied, which leach into the groundwater and can also be found in run-off waters[4]. Due to the mentioned toxicological effects, sensitive and reliable analytical methods are needed for the determination of trace amounts of these compounds[5]. Levofloxacin is a broad-spectrum antibiotic that prevents the growth of bacteria, so it is classified as a bacteriostatic[5, 6]. It is widely used in the treatment of patients with Covid-19 in recent two years[7]. Several methods have been applied for determining levofloxacin residues

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in aqueous samples, including, solid-phase microextraction (SPME)[8, 9], dispersive-solid phase extraction[10, 11], molecularly imprinted polymer (MIP)[12, 13]and other techniques based on microextraction principles[14, 15]. The pipette tip (PT) is a micro-scale of SPE that is used for the separation and extraction of environmental pollutant samples[16]. This method used small volume and low consumption of any solvents that satisfied by green chemistry rules. It differs from common SPE in that a small amount of sorbent is inserted into a pipet tip, and it is relatively inexpensive without a special auxiliary device for extraction. In general terms, an advantage of pipet tips for sample preparation is that extraction can be carried out faster and more facile than conventional SPE cartridges. Many SPE methods based on adsorbents (silver nanoparticles (AgNPs) coating on micro glassy balls (MGB), graphene oxide-packed micro-column, magnetic nanoparticles, cadmium Sulfide Nanoparticles, amine-functionalized bimodal mesoporous silica nanoparticles) were used for extraction heavy metals, organic materials and drugs in different matrixes such as, water, human, vegetable, food and drug samples [17-22]. In recent years, many types of research have been focused on the synthesis and application of metal-organic framework (MOF) compounds. The MOFs are one of major approaches for making sorbents with large surface area. MOFs are a new class of porous compounds consisting of organic linkers coordinated to inorganic metal nodes[23]. Thermal and chemical stability, porosity, their tailorable structures and properties are some advantages of MOFs for several purposes such as separation, sensing drug delivery and the removal of toxic materials from air and water[24, 25]. In this work we used cobalt metal-organic framework(Co-MOF) for the preparation of a novel solid-phase adsorbent. Then pipette–tip solid-phase extraction methods were used and developed for preconcentration and extraction of levofloxacin.

2. Material and methods

2.1. Reagents

All chemicals were of analytical grade. Cobalt nitrate ($Co(NO_3)_2.6H_2O$) and pyridine 2,6-dicarboxylic acid (98%) were purchased from Aldrich (Millipore-Sigma, USA). Ethanol and N,N-dimethyl formamide (DMF) were obtained from Merck KGaA (Germany). Other reagents such as the hydrochloric acid (HCl, CAS N: 7647-01-0,), the sulphuric acid (H_2SO_4, CAS N: 7664-93-9) with high purity were purchased from Sigma, Germany. The pH of the samples is tuned by the sodium acetate (CH_3COONa/ CH_3COOH), the sodium phosphate (HPO_4^{2-}/ NaH_2PO_4 -) and the ammonium buffer for pH of 4-5, 5-8 and pH 8-10, respectively. All the plastic and glass tubes were put on the HNO_3 solution (0.5 M, v/v) for at least 2 days and then washed with DW many times.

2.2. Instrumental analysis

The chromatographic analysis was carried out on Cecil HPLC system (Cecil, England), equipped with a UV detector. A reverse-phase ACE-C18 column (250 mm × 4.6 mm i.d.) was used for separation of the analyte. The mobile phase was a mixture of trimethylamine phosphate buffer (1%, Ph=4.30) and acetonitrile (12/88, v/v) at a flow rate of 1.0 mL min-1. The column temperature was kept at 30oC and the detection wavelength was set at 280 nm. The injection volume was 10 µL.

2.3. Synthesize of Co-MOF

Synthesize of adsorbent was described in one of our previously reported works[26]. First, 1.85 mmol of pyridine 2,6-dicarboxylic acid and 5.62 mmol of cobalt nitrate were dissolved in 14 mL of ethanol. After this, the solution was transferred into a Teflon reactor with a tight cap and kept for 7 hours at 85oC. The products were washed with dimethyl formamide (DMF). After mixing and dissolving the reactants, the clear solution radiated in the ultrasound bath for 13 min at working conditions of 160 W, 1 kJ and 21 kHz. Synthesized Co-MOF was stored at 4 oC.

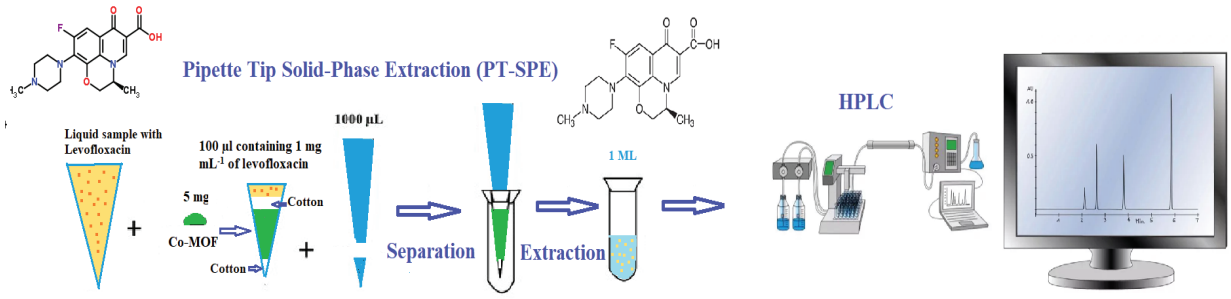

Fig. 1. The levofloxacin determination based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE)

2.4. Pipette- tip solid phase extraction procedure

5 mg of Co-MOF was put into the pipette-tip. The extraction of levofloxacin was performed by attaching this pipette-tip extractor to a variable pipette. Then 100 µl of the aqueous standard solution containing 1 mg mL-1 of levofloxacin in a 5 mL glass test tube was withdrawn into the sorbent and dispensed back into the same tube. Before the optimization of the number of draw/ eject cycles, the extraction was optimized with 10 cycles. The adsorbed levofloxacin on the surface of the Co-MOF was eluted with 1000 µl of a mixture of methanol-acetonitrile (90:10, v/v) into a 2-mL glass vial, also with 10 aspirating/ dispensing cycles (Fig.1). So, the levofloxacin was determined based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE).

3. Results and discussion

3.1. Optimization of extraction conditions

For achieving the highest extraction efficiency, several parameters that influenced the extraction procedure were investigated and optimized as follows.

3.1.1.Type of eluting solvent

Several solvents including, methanol, acetonitrile, deionized water, mixture of water- acetonitrile and mixture of methanol- acetonitrile were investigated for obtaining the best extraction efficiency. With different polarities were evaluated to desorb levofloxacin from the Co-MOF sorbent. Experiments showed that the levofloxacin was completely desorbed by a mixture of methanol and acetonitrile (90:10, v/v)

3.1.2.Effect of pH

The effect of sample pH on the recovery of levofloxacin was investigated between 2.0 and 10.0, using a one molar NaOH or HCl. As shown in Figure 2, a pH value of 7.0 has highest extraction efficiency. However; in stronger acidic and basic media, extraction efficiency was decreased.

3.1.3.Effect of amount of sorbent

To obtain high extraction efficiency with good recoveries of levofloxacin, the amount of sorbent for pipette-tip extraction was changed between 1-5 mg. The adsorption ability of Co-MOF was increased by increasing amount of nanocomposite up to 2 mg. After that, the extraction recovery became constant and hence, amount of adsorbent was optimized at 2 mg (Fig. 3).

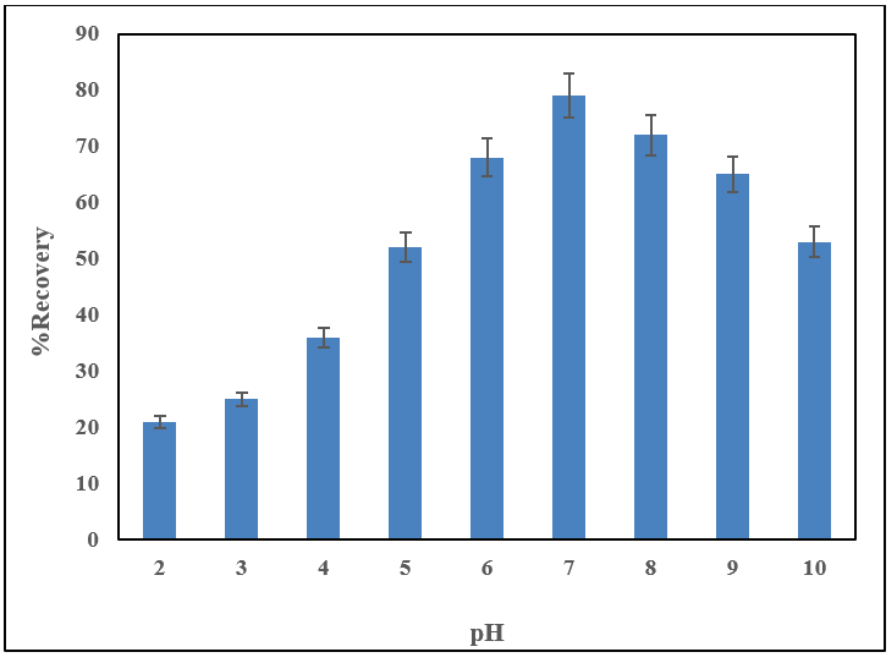

Fig. 2. Effect of pH on recovery of levofloxacin by pipette-tip solid-phase extraction technique

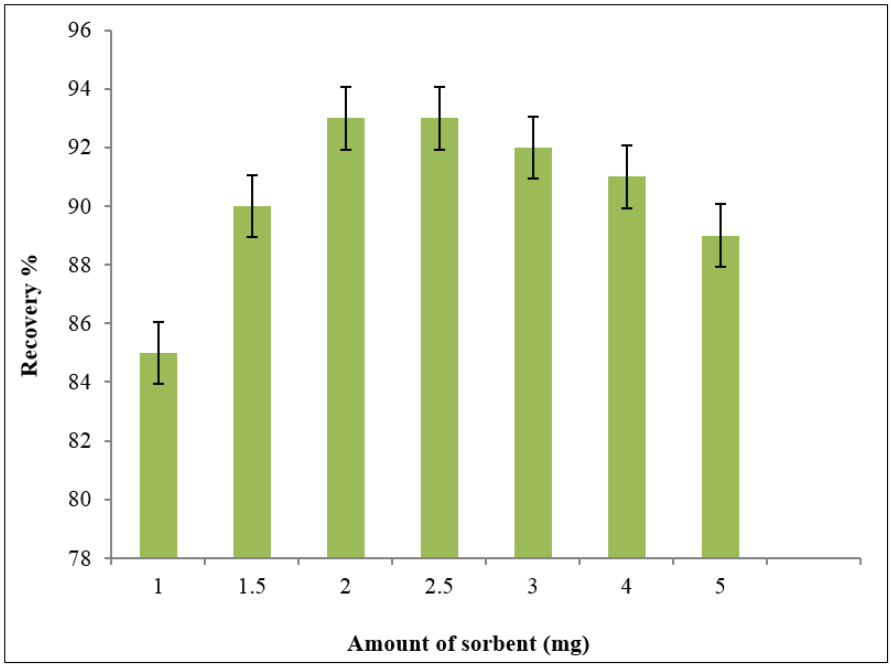

Fig.3. Effect of amount of adsorbent on recovery of levofloxacin based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE)

3.1.4.Effect of volume of eluting solvent

We tried to obtain the smallest volume of eluent solvent which provides the highest enrichment factor. Volumes between 5 to 50 µL of a mixture of methanol- acetonitrile were examined. As shown in Figure 4, between 15 and 30 µL of the eluting solvent, the recovery of levofloxacin is at its highest value, which means a larger volume of eluting solvent provides a better elution. Therefore, the eluting volume of 20 µL was selected for further experiments.

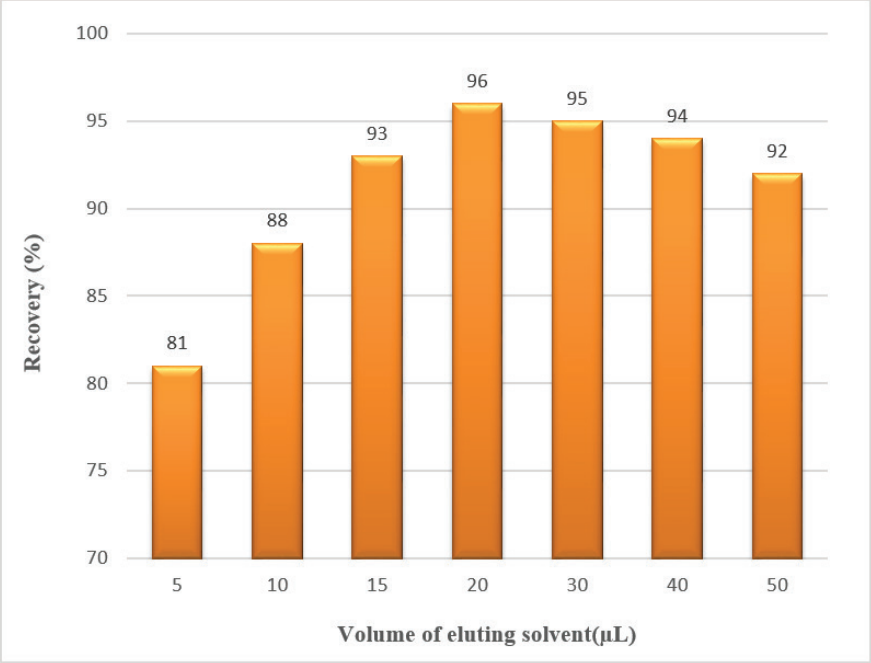

Fig. 4. Effect of eluent volume on recovery of levofloxacin based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE)

3.1.5. Effect of volume of sample solution

The volume of sample solution is an important factor in pipette- tip solid phase extraction. Different volumes of sample solution were evaluated in the range of 300 to 5000 µL. Figure 5 shows that with increasing the sample solution, extraction recovery of levofloxacin is also increased up to 3800 µL. Hence, this point of sample solution was selected as optimized volume. So, considering 20 µL of eluent, and extraction efficiency of 100 %, a preconcentration factor of 200 was achieved

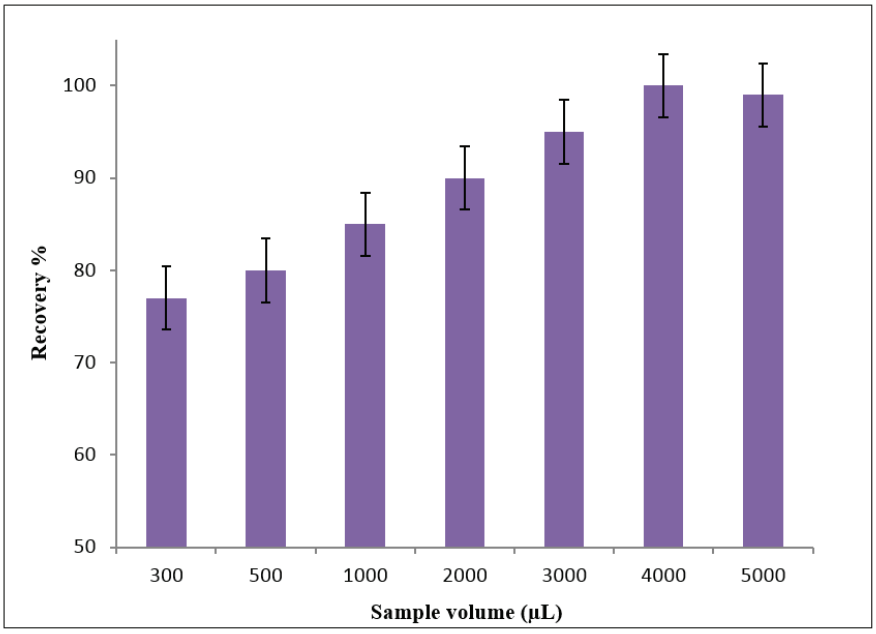

Fig. 5. Effect of sample volume on recovery of levofloxacin based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE)

Table 1. Analytical performance of proposed PT-SPE method

Parameter	Analytical feature
Dynamic range (µg L^{-1})	0.70-150
Repeatability	0.99
Repeatability[b] (RSD %)	2.75
Limit of detection (µg L^{-1})	0.041
Enrichment factor (fold)	200
Total extraction time (min)	≤ 10

3.1.6.Effect of number of aspirating/dispensing of sample

The number of aspirating/dispensing cycles is another important in the pipette-tip extraction. The time of extraction depends on the number of cycles and the volume of solution that passed through the extractor. The results show that the highest extraction efficiency for levofloxacin was 10 cycles, while using a 4000 µL of the sample. At a higher number of cycles, the back extraction of analytes from adsorbent into the sample solution might occur, causing a decrease in the recovery. The optimal number of aspirating/dispensing cycles used for desorption of levofloxacin was found to be 11 cycles at 9.5 min.

3.2. Analytical performance

The analytical performance of the suggested pipette-tip extraction coupled with HPLC–UV was investigated, and the results are summarized in Table 1. Limit of detection (LOD) was obtained based on a signal-to-noise ratio of 3. The linearity range was studied by varying the concentration of the standard solution from 0.1 to 200 µg L^{-1}. The repeatability of the method, expressed as relative standard deviation (RSD), was calculated for seven replicates of the standard at an intermediate concentration (100 µg L^{-1}) of the calibration curve.

3.3. Application of proposed method in real samples

The suggested pipette-tip procedure was applied for three hospital wastewater real samples. All samples were filtered through 0.45 µm nylon membranes before analysis and were sonicated for 10 min and the slurry was centrifuged at 3500rpm for 5 min. The extraction procedure was repeated three times on each wastewater sample. For evaluation of the analytical performance of the proposed method in reaa samples, the samples were spiked with 3 different concentrations to investigate the matrix effect on its determination. The results are shown in Table 2. As can be seen, recoveries of are adequate; therefore, we can justly this assumption that matrix effect is negligible for the analysis of the target analyte.

4. Conclusion

In this study, pipette-tip solid phase extraction with a novel sorbent based on Co-MOF followed by high performance liquid chromatography (HPLC) has been developed for the determination of levofloxacin in wastewater samples. Due to very high surface areas and short diffusion rate, high adsorption capacities can obtain in a very short time at pH=7. The optimized method is found to be fast, economical, sensitive, accurate and simple. The LOD, linear range (LR) and mean absorption capacity of Co-MOF was obtained 0.041 µgL^{-1}, 0.1 to 200 µgL^{-1} and 156.7 mg g^{-1}, respectively for 10 analyses. The range adsorption capacity of Co-MOF adsorbent for levofloxacin was obtained at 114.9-176.8 mg g^{-1}. Therefore, the efficient extraction and separation of levofloxacin in water and wastewater samples were obtained by the pipette tip solid-phase extraction procedure (PT-SPE) before being determined with HPLC.

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Table 2. Validation of proposed method in real hospital wastewater based on Co-MOF adsorbent by pipette tip solid-phase extraction procedure (PT-SPE)

Wastewater Sample #	Added ($\mu g.L^{-1}$)	Found ($\mu g.L^{-1}$)	Recovery (%)	RSD % (n=7)
Sample 1	0	25.0	-	-
	50	89.1	98.2	3.5
	100	124.3	74.3	4.2
Sample 2	0	41.0	-	-
	50	89.2	98.7	1.3
	100	140.1	98.1	2.5
Sample 3	0	55.0	-	-
	50	104.01	98.7	4.1
	100	154.2	98.5	5.6
Sample 4	0	95.5	-	-
	50	147.4	103.8	2.8
	100	192.1	96.6	3.3
Sample 5	0	0.58	-	-
	0.5	1.06	96.0	2.7
	1.5	2.11	102.0	3.9

6. Conflict of Interest

The authors have declared no conflict of interest.

7. References

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