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Quantitative Determination of Fluoroquinolones in Contaminated Soils by HPLC with Solid-Phase Extraction

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Abstract:

This work reports the development of an analytical method for the simultaneous analysis of three fluoroquinolones; ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL) in soil matrix. The proposed method was performed by using microwave-assisted extraction (MAE), solid-phase extraction (SPE) for samples purification, and finally the pre-concentrated samples were analyzed by HPLC detector. In this study, various organic solvents were tested to extract the test compounds, and the extraction performance was evaluated by testing various parameters including extraction solvent, solvent volume, extraction time, temperature and number of the extraction cycles. The current method showed a good linearity over the concentration ranging from 1 – 300ng g^{-1} with correlation coefficients (r^2 > 0.998) for all test compounds. Good recoveries were also obtained for the test compounds ranged from 89 – 99 %. The relative standard deviation (RSD) from intra-day and inter-day precision was lower than 7%, and the detection (LOD) and quantification (LOQ) limits ranged from 0.9 – 2.7ng g^{-1}.Finally, the proposed method was applied to determine the target compounds in real soil samples irrigated with wastewater. The results confirm the presence of the test compounds in soils with values ranging from 1.9 – 4.6ng g^{-1}, as well as the suitability of the proposed method to determine the test compounds in real soil matrix as well as the ability of the test compounds to accumulate in soils irrigated with sewage.

Key words: Contaminated soils, Fluoroquinolones, HPLC, Microwave-assisted extraction, Solid-phase extraction.

Introduction:

Pharmaceuticals are the most common practice for human treatment and/or prevention of illness. In recent years, the developments in pharmacology have made the pharmaceuticals as one of the important group of contaminants in the environment and recognized as an emerging research area in the environmental chemistry (1-3). Antibiotics are one of the important compounds used for human and veterinary medicine which represent a source of concern to the scientists.

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These compounds can enter the terrestrial environment as a result of many sources such as industrial processes, excretion via urine and feces as conjugated forms, as bioactive metabolites, or through the application of animal manure to the agricultural farmlands (4,5).The presence of antibiotics in high levels is negatively affecting the environment community by their chronic effect on human health, animals and terrestrial living organisms (6).

Fluoroquinolones used for humans and animals treatment have been considered as one of the important class of antibacterial agents due to their efficient activity against many types of bacteria by inhibiting the DNA gyrase enzyme which responsible of DNA replication (7). The chemical structure of fluoroquinolones is derived from quinolone which has a bicycle structure with nitrogen, carboxylic acid and carbonyl groups in positions 1, 3 and 4, respectively. In addition to these functional groups, fluoroquinolones contain also fluorine in position 7 which contributes to

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carboxylic group in polar character of fluoroquinolones (log K_{ow} between -1.03 to 0.89). The presence of ketone, hydroxyl group and amid in fluoroquinolones structure making it able to form complexes with many of divalent and trivalent cations such as Mg^{2+}, Ca^{2+}, and Al^{3+} which make fluoroquinolones capable of being adsorbed on soil clay surface (8,9)

The extraction of pharmaceuticals via extraction techniques from solid samples has been considered more complicated as compared to the extraction from aqueous samples due to the potential interferences. Thus, due to the ability of organic matter to combine or interact with several types of antibiotics as well as the adsorption capacity of antibiotics on clay surface, the extraction method might give a weak reproducibility in comparison to aqueous samples (10,11). In fact, there are several parameters that influence the efficiency of extraction method to extract a target pharmaceutical as compared to the other methods. These parameters include mainly the type of chemical solvents, time of extraction, temperature, as well as the number of extraction cycles (12,13).

From the literature, the pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), and microwave assisted extraction (MAE) are considered as the most important extraction procedures applied for pharmaceuticals extraction from soils and sediments. These types of extraction were followed by a clean-up step with solid-phase extraction (SPE) using many types of reversed phases such as Oasis Hydrophilic Lipophilic Balance (HLB), C18, and polymeric phase (14-17).

Depending on literature, only limited methods have been developed for the quantification and determination of antibiotics in soil matrices (8). Therefore, the objective of our study is to develop an analytical method using microwave-assisted extraction (MAE) followed by solid phase extraction (SPE) and HPLC for simultaneous determination of three fluoroquinolones: ciprofloxacine, norfloxacine and ofloxacin in soil samples. The effects of variables; type of solvent, time of extraction, number of extraction cycles and temperature were investigated.

Materials and Methods:

Soil sampling

Soil samples were collected from the top layer (0 – 20cm) of a garden soil located at Jadidat Al-Shat district (Diyala, Iraq) that has never been treated before with the wastewater or animal manure. The soil sample was transferred to the laboratory with polyethylene bags to avoid possible contaminants and interferences. In the laboratory, the soil was left to dry at room temperature for 72h, homogenized, passed through 2mm sieve and then stored in room temperature. Before spiking with the tested pharmaceuticals, the main physico-chemical characteristics of the soil samples were determined according to standardized procedures (AFNOR 1999) and (ISO 1999) procedures. The pH of the test soil was determined in distilled water extracts (1:2.5 w/v) according to (NF ISO 10390 (2005)). The organic matter was determined by the method of loss of weight on ignition (LOI). The $CaCO_3$ was estimated by titration method (18). The main physical-chemical properties of the test soil were: pH= 7.73 ± 0.1, organic matter (8.3 ± 0.11), $CaCO_3$ (33 ± 2.3), and sand 28%, silt 20% and clay 52%.

Chemicals and reagents

The pure standards of the test compounds (Table 1) ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL) with high purity grade (>99%) purchased from USP were kindly supplied by a local pharmaceutical industries.

All organic solvents with HPLC grade used in this study including acetonitrile, methanol and acetone were obtained from Sigma-Aldrich. Disodium-ethylenediaminetetraacetate (Na_2EDTA) was obtained from Sigma-Aldrich (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q device from Millipore system. The stock solutions of the test compounds were prepared from dissolving of appropriate amount of the pure standards in methanol. Working solutions were prepared by dilution of the stock solutions in methanol.

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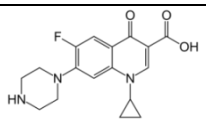
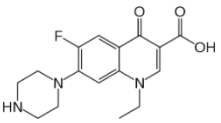
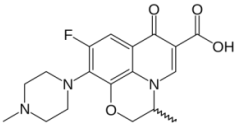
Table 1. Physico-chemical properties of the test pharmaceuticals (19, 20)

Compound	M.W	log *K*ow[a]	pKa[b]	Chemical structure
Ciprofloxacine	331.3	0.28	pK_{a1}= 5.90 pK_{a2} = 8.89	
Norfloxacin	319.3	-1.03	pK_{a1}= 6.23 pK_{a2} = 8.55	
Ofloxacin	361.3	-0.39	pK_{a1}= 5.97 pK_{a2} = 8.28	

[a]Logarithm of the octanol–water partition coefficient.
[b] Acidic ionization constant.

Sample preparation

The microwave-assisted extraction (MAE) was performed by using a microwave oven (Multiwave 3000; Anton Paar GmbH, Germany). 1gm of soil was placed into extraction vessel, followed by 0.25g Na_2EDTA, and 10ml of the extraction solution (40:40:20 acetone: methanol: formic acid) was added. The extraction process was performed at the temperature of 80°C at 500 watt for 15min. The microwave vessels cooled to room temperature. After cooling, the extracts were centrifuged for 15min at 4000rpm. The resulting supernatants were filtered through 0.45µm, evaporated down to approximately 5ml, and then the extract was supplemented with Milli-Q water to 500ml before the clean-up step.

After microwave extraction, the clean-up step was performed by solid phase extraction (SPE) using Oasis HLB cartridges [poly(divinylbenzene-co-Npyrrolidone)](Waters Corporation, Millford, MA, USA) (21, 22). An aliquot of the extract was passed through HLB cartridges at a rate of 1ml.min^{-1} using a vacuum system. The cartridge was previously performed with 5ml of methanol and followed by 5ml of Milli-Q water. The cartridges was subsequently washed twice with 5ml of Milli-Q water, dried for 15min under a gentle N_2 steam, and then eluted by 5ml of methanol. The eluted volume was condensed to a final volume of 2ml under a gentle N_2 steam, and then analyzed by HPLC.

To optimize the type of solvents, 1gm of uncontaminated soil samples, spiked with a mixture of test pharmaceuticals at 200ng g^{-1}, left for 48h to equilibrate and to allow the solvent evaporation. Five different solvent mixtures were chosen: acetone: H2O (50:50), methanol: H_2O (50:50), acetonitrile: H_2O (50:50), acetone: methanol (50:50) and acetonitrile: methanol (50:50). These solvent solutions were mixed with the spiked soil samples and extracted with microwave oven. After extraction the supernatant was filtered and evaporated to a volume of 200µL, and then the final volume was reconstituted with 10ml of Milli-Q water. The reconstituted sample was purified using solid phase extraction, and then measured by HPLC.

Analysis by HPLC

The determination of the test compounds was performed using an Agilent HPLC system (1100 HPLC system) equipped with Binary pump 600 bars, vacuum degasser and oven temperature of 25 °C. The separation of test compounds was performed using XDB-C18 (100 mm × 4.6 mm, 3.5 µm) column from Agilent Technologies.

The mobile phase used in this study was composed from solvent A: Milli-Q water acidified with a solution of 0.1% formic acid, and B: methanol acidified with 0.1% formic acid with elution gradient of 40% of solvent A from 0.01 to 1.0 min, and from 40% to 80% of solvent A in the next 2 min., then back to 40% of solvent A for 5min. Before the next sample injection, the column equilibrating with 100% (B) for 5min., the flow rate of analysis was 0.2ml/min and the injection solution was 20μL. The UV detector signal was monitored at 280nm.

Results and Discussion:

Optimization of the extraction procedure

The extraction of fluoroquinolones from soil matrix requires exhaustive conditions as compared to aqueous solution, since these pharmaceuticals bind strongly to the soil constituents (23, 24). Therefore, to evaluate the performance of extraction technique to isolate the test compounds from soil matrix, several parameters influencing the MAE performance should be taken in consideration.

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Depending on literature, the main influencing parameters include: the nature of the extraction solvents and its volume; the microwave irradiation time; the extraction temperature as well as the number of the extraction cycles (23, 24).

Based on the physico-chemical properties (amphoteric properties) of the test compounds and the presence of carboxylic acid group (pKa 5.5–6.3) and amino functional groups (pKa 7.6–9.3) in their chemical structures, different solvent mixtures with different pH values (acidic and basic pH) were chosen in order to select an appropriate extractant for extraction of the test compounds in this experiment.

Since the fluoroquinolones have a tendency to form complexes with metal ions reducing the extraction efficiency, Na2EDTA was added to samples to avoid test compound bonding with metal residues in the sample which reduce the extraction efficiency (25, 26, 27).

Figure.1 shows that the better extraction yield for test pharmaceuticals was obtained with the solvent mixtures of acetone: H_2O and MeOH:H_2O with extraction recovery reached 70% for the both solvent mixtures in comparison to the other test solvents.

Evaggelopoulou et al. (28) have demonstrated that the addition of an amount of formic acid resulting in better extraction yields. This result led to test more complex organic solvent mixtures based on the addition of formic acid since the test compounds presented higher extraction efficiency at low pH values due to the electrostatic repulsion between the test compounds and the soil clay surface. Therefore, it has been decided to add a different volume of 10% formic acid (v/v) to a various volume of acetone: MeOH mixtures.

Figure. 2 presents the extraction recoveries for the test analytes obtained from different compositions ratio of the solvent mixture (acetone: MeOH:formic acid).

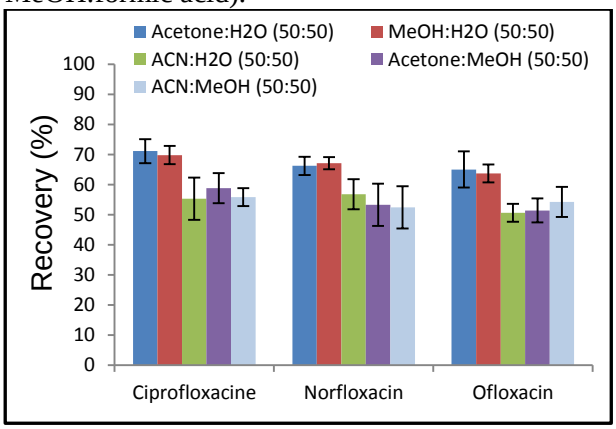

Figure 1. Recoveries (n=5) of the selected pharmaceuticals with various types of extraction solvents

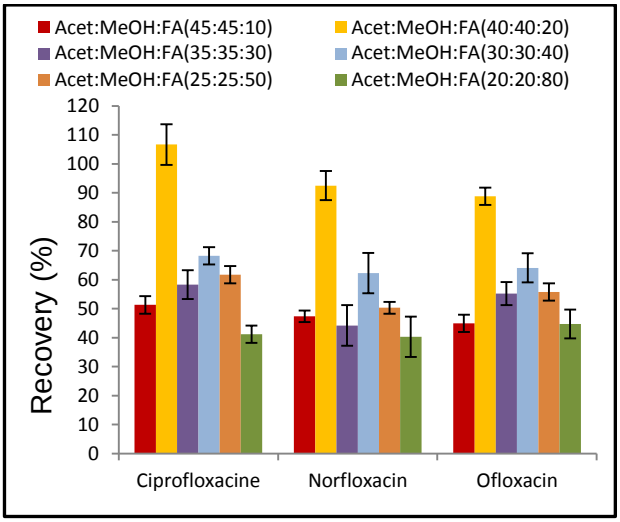

Figure 2. Recoveries (n=5) of the selected pharmaceuticals with various compositions of the solvent mixtures (acetone: MeOH: formic acid).

As shown in Fig. 2, the composition of (40:40:20) showed the better extraction result with recoveries ranged between (88 – 106%) as compared to the other solvent compositions, with differences between the test compounds recoveries depending on their polarity.

One of the significant parameters influencing the extraction yield is the volume of solvent solution. For the optimization of this variable, a series of different volume (5, 10, 15, 20, 25, 30 ml) of the solvent mixture (acetone: MeOH:formic acid (40:40:20) was investigated. The results showed that 10ml of extractant resulted in a maximal and unchangeable extraction yield which was in agreement with many studies which demonstrated the efficiency of the selected time on extraction yields (29, 30). As a result, 10ml of extractant was adopted for the following analyses.

Therefore, to investigate the effect of the extraction time as well as the number of extraction cycles on the extraction yields of the test compounds, six different times (5, 10, 15, 20, 25 and 30 min) and three static cycles were examined in combination with different microwave power ranged from 100 to 800W. The results are shown in Fig. 3 and 4. It is showed that the extraction recoveries were increased with the increase of extraction time until 15min, and then decreased when the time exceeded 15min towards 30min. With the selected extraction time, the results showed that two extraction cycles were found necessary for quantitative extraction of the test compounds.

On the other hand, among the tested microwave power, the irradiation power of 500W exhibited the best extraction yields. These results

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indicate that the combination of long times with high microwave power lead to reduce the HPLC signal for the test compounds, which can be explained by the decomposition of the test compounds under these conditions (30). Therefore, the 15min of extraction and the 500W of irradiation power were selected to augment the extraction yields and to prevent the decomposition of the test compounds.

The temperature has an important role on the extraction efficiency due to its effect on the solubility of the target analytes (31). For this reason, we studied the effect of temperature on the recovery of the test compounds by varying the microwave oven temperature from 30°C to 100°C. The results of the temperature effect are shown in Fig. 5. It can be seen from Fig. 5 that the higher extraction yielded for the test compounds were obtained when the temperature increased from 30°C to 80°C, and then it decreased with the increasing of the temperature.

The increase of extraction efficiency between 30 to 80°C might be attributed to the increasing of the extraction kinetics and subsequently increase the solubility of the target compounds. On the other hand, when the temperature exceeded 80°C, the extraction efficiency decreased, which might be attributed to the thermal degradation processes or the ability of the extraction method to extract the soil constituents with high temperature (32**)**.

Therefore, 80°C was selected to be the feasible extraction temperature in this study. As mentioned above, the extreme extraction conditions concerning the optimization of the MAE referred to the strong binding of the test compounds with the soil constituents. However, despite these drastic conditions, the extraction recoveries reached more than 80% for all test compounds.

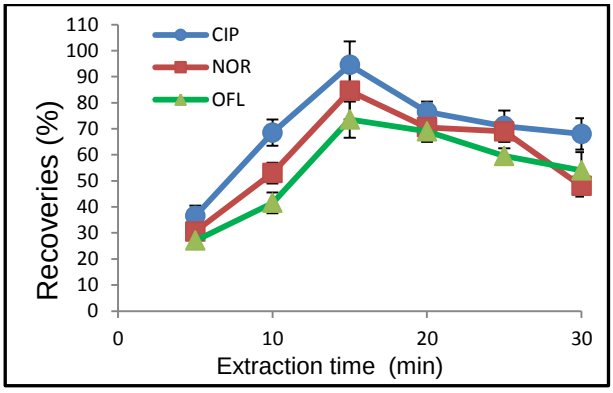

Figure 3. The effect of the extraction time (n=5) on the extraction efficiency of the selected pharmaceuticals by the solvent composition (acetone: MeOH: formic acid).

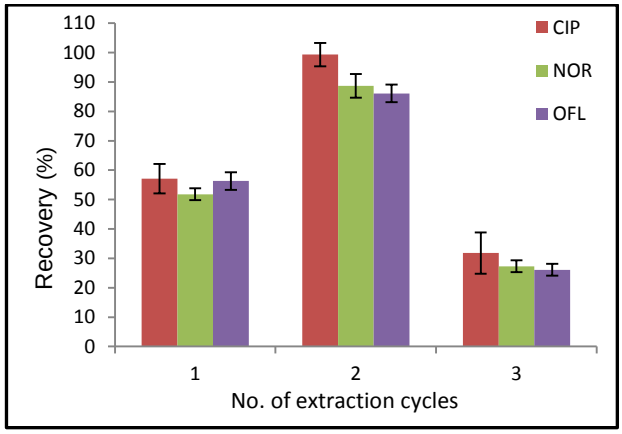

Figure 4. The effect of the extraction cycles (n=5) on the extraction efficiency of the selected pharmaceuticals by the solvent composition (acetone: MeOH: formic acid).

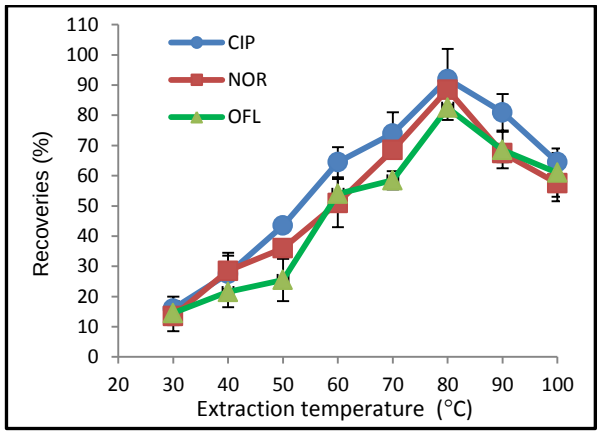

Figure 5. The effect of the extraction temperature (n=5) on the extraction efficiency of the selected pharmaceuticals by the solvent composition (acetone: MeOH: formic acid).

The figure 6 represents the HPLC chromatograms for the spiked soils with 25ng g^{-1} of the studied compounds, which illustrate narrow peak shape, indicating that the studied compounds were efficiently separated and there were no interferences such as the organic matter observed.

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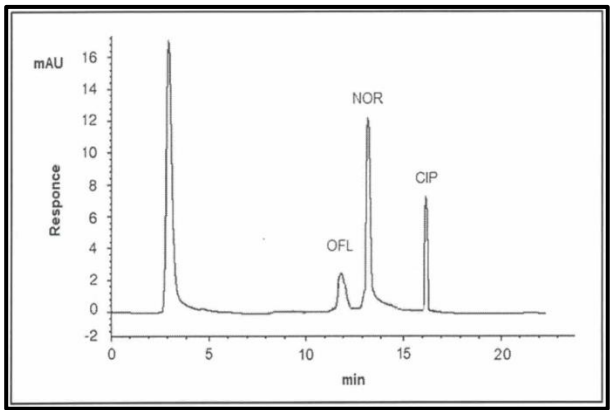
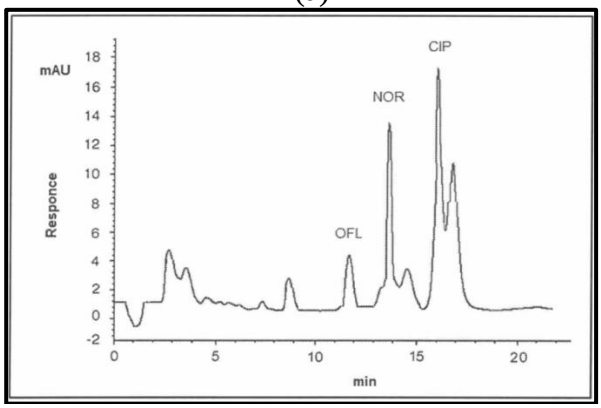


(a)



(b)

Figure 6: The HPLC chromatograms of a standard mixture of the studied compounds (a) and an extract of soil spiked with a mixture of 25ng g^{-1} of each compound (b).

Analytical performance

In order to assess the reliability, feasibility as well as the analytical errors of the current method, the method performance was evaluated in terms of linearity, precision, selectivity, recovery and limits of detection and quantification. To evaluate these parameters, the soil samples were analyzed before spiked with the test compounds (CIP, NOR and OFL), and the results showed that the soil samples were free of these test compounds.

Linearity

To assess the method linearity, an external calibration curves were performed using soil samples spiked with six different concentrations of the test compounds over the concentration range from 1 – 300 ng g^{-1} and these tests were performed in five replicates. The construction of the calibration curve was made according to the relationship between the signal response and the concentration of the target compounds in order to obtain the linear regression equation.

The results showed that the calibration curves were linear over the studied concentration range for all the test compounds with coefficient regression (r^2) in the range of 0.9969 – 0.9998 (Table 2).

Recovery

The soil samples used to perform the recovery of the current method were previously analyzed to ensure the absence of the target compound from these samples. The recovery test which represents the efficiency of the proposed method was performed by spiked the soil samples with a mixture of the test compounds at three spiking levels: 25, 50 and 200ng g^{-1} by adding 5ml of the corresponding standard solution to the soil samples and left for 24hr before analysis. The recovery tests were performed in five replicates (n=5).

It can be seen from Table 2 that the proposed method is capable of determining the test compounds at all the concentration levels with a recovery percentage ranged from 93.3 – 99.2%, 89.1 – 96.7% and 90.9 – 93.5% for CIP, NOR and OFL respectively with relative standard deviation (RSD) lower than 12. The results obtained confirmed the efficiency and the suitability of the proposed method to analyze the test compounds in soil matrix.

Table 2. Linearity and recovery (n=5) for the test pharmaceuticals in soil.

Compound	Linear equation	Linearity (r^2)	Recovery (%) Spiked level (ng g^{-1})		
			25	50	200
Ciprofloxacine	y= 1.2883x + 0.0465	0.9984	93.3±11	99.2±5	96.6±8
Norfloxacin	y= 2.1634x + 0.198	0.9998	91.7±4	96.7±7	89.1±4
Ofloxacin	y= 1.9523x + 0.0528	0.9986	91.2±9	93.5±4	90.9±7

Selectivity

It is well known that the matrix effect of the solid samples (soil or sediment) should be taken into consideration when analyzing the pharmaceutical compounds. Although the selectivity and sensitivity of the analytical techniques were considered, the presence of different substances (metals, impurities and organic matter) in soil samples influenced (increase or decrease) the signal response of the target compounds in comparison to the internal standard in the same matrix. For this reason, the selectivity of the current method was performed by the analysis of blank samples and the chromatograms

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obtained from blank samples and from spiked blank samples with the target compounds were compared.

The results showed that no chromatographic peak was found in the same retention time of the selected compounds, indicating the absence of the matrix effects on the signal intensity of the test compounds in the blank samples and proving the selectivity of the current method.

Limits of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) which represent the selectivity of the analytical method were determined based on the signal-to-noise –ratio (S/N). LOD and LOQ were determined at 3 and 10 times the signal of the baseline noise obtained from blank soil samples measured at the retention times of the target compounds.

The result obtained showed that the LOD values ranged from (0.9 – 1.88ng g^{-1}), whereas LOQ values ranged from (1.7 – 2.7ng g^{-1}) (Table 3). As shown in Table 3, the current method can be considered satisfactory since the LOD and LOQ values were in the range of detection limits provided by other researchers for the determination of these compound residues in soil matrix.

Table 3. limits of detection and quantification (LOD and LOQ), and precision (relative standard deviation, RSD %) for the test pharmaceuticals in soil.

Compound	LOD (ng g^{-1})	LOQ (ng g^{-1})	Precision (RSD %)	
			Intra-day	Inter-day
Ciprofloxacine	1.88	2.7	1.6	2.3
Norfloxacin	1.01	1.9	2.3	3.9
Ofloxacin	0.9	1.7	2.7	6.7

Precision

The precision of the current method was assessed in terms of intra-day and inter-day by spiked the soil samples with three concentration levels (100, 200 and 500ng g^{-1}) of the test compounds in five replicates. The precision of the of the spiked soil samples which expressed as the relative standard deviation (R.S.D %) was determined during the same day and in eight different days. The RSD values ranged from 1.6% – 6.7% (Table 3), indicating the good repeatability and reproducibility of the proposed method for the analysis of the target compounds since the obtained RSD values is less than 18% (8, 33).

Analysis of environmental soil samples

The proposed method was applied for the analysis of five agricultural soils located at Jadidat Al-Shat district (Diyala, Iraq) irrigated with Tigris river water which contains sewage. These soils were used to cultivate some of the food croup like lettuce, tomato and cabbage. The results obtained are listed in Table 4. As can be seen, the results showed that the target compounds were detected at low levels in some soil samples. The ciprofloxacine was detected in four of five samples at levels ranged from 2.4 – 4.6ng g^{-1}, whereas, norfloxacine and ofloxacine were detected in two of five samples with levels not exceeded 2.5ng g^{-1}, indicating that ciprofloxacine is the dominate antibiotic in soils.

Table 4. Concentration of the test pharmaceuticals in real soil samples (ng g^{-1} dry matter).

Compound	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Ciprofloxacine	3.4	2.7	< LOQ	3.9	4.6
Norfloxacin	Nd*	2	< LOQ	2.07	Nd
Ofloxacin	Nd	1.9	Nd	2.1	< LOQ

*Nd: not detected

Conclusion:

Our study deals with the validation of a simple, accurate and sensitive analytical method for simultaneous determination of three fluoroquinolons in soil matrix. The proposed method involves the isolation of the test compounds from soil by using the MAE, purified by SPE and then analyzed by HPLC.

The MAE-SPE-HPLC method provides good recoveries, low detection and quantification limit values, high correlation coefficient, as well as high precision expressed by a relative standard deviation (RSD) which is lower than 7%. These results confirm the efficiency of the proposed method for the analysis of the test compounds and its capability to diminish the effect of the interferences that presence in soil matrix on the extraction efficiency.

The suitability of the proposed method for the routine quantitative determination of the test compounds is tested by applying it to a real agricultural soil samples that already received wastewater. The results demonstrate that the water containing sewage can be considered as an important source for antibiotics contamination.

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Conflicts of Interest: None.

References:

1. Odendaal C, Seaman MT, Kemp G, Patterton H, Patterton HG. An LC-MS/MS based survey of contaminants of emerging concern in drinking water in South Africa. S. Afr. J. Sci. 2015; 111 (9-10): 1–6.
2. Chuang YH, Zhang Y, Zhang W, Boyd SA, Li H. Comparison of accelerated solvent extraction and quick, easy, cheap, effective, rugged and safe method for extraction and determination of pharmaceuticals in vegetables. J. Chromatogr. A. 2015; 1404:1–9.
3. Madikizela LM, Ncube S, Chimuka L. Uptake of pharmaceuticals by plants grown under hydroponic conditions and natural occurring plant species: A review. Sci. Total Environ. 2018; 636 (15):477–486.
4. Li C, Chen J, Wang J, Ma Z, Han P, Luan Y, Lu A. Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci. Total Environ. 2015; 521–522:101–107.
5. Fijalkowski K, Rorat A, Grobelak A, Kacprzak MJ. The presence of contaminations in sewage sludge – the current situation. J. Environ. Manag. 2017; 203:1126–1136.
6. Frade VMF, Dias M, Teixeira ACSC, Palma MSA. Environmental contamination by fluoroquinolones. Braz. J. Pharm. Sci. 2014; 50(1): 41-54.
7. Bisacchi GS. Origins of the Quinolone Class of Antibacterials: An Expanded "Discovery Story," J. Med. Chem. 2015; 58(12):4874–4882.
8. Salvia MV, Fieu M, Vulliet E. Determination of Tetracycline and Fluoroquinolone Antibiotics at Trace Levels in Sludge and Soil. Appl. Environ. Soil Sci. 2015:1-10
9. Cuprys A, Pulicharla R, Brar SK, Drogui P, Verma M. Fluoroquinolones metal complexation and its environmental impacts. Coord. Chem. Rev. 2018; 376 (1):46-61.
10. Chen G, Liu X, Tartakevosky D, Li M. Risk assessment of three fluoroquinolone antibiotics in the groundwater recharge system. Ecotoxicol. Environ. Saf. 2016; 133:18–24.
11. Miller EL, Nason SL, Karthikeyan K, Pedersen JA. Root uptake of pharmaceuticals and personal care product ingredients. Environ. Sci. Technol. 2016; 50(2):525–541.
12. Radjenovic J, Jelic A, Petrovic M, Barcelo D. Determination of pharmaceuticals in sewage sludge by pressurized liquid extraction (PLE) coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Anal. Bioanal. Chem. 2009; 393 (6-7):1685–1695.
13. Aufartová J, Brabcová I, Torres-Padrón ME, Solich P, Sosa-Ferrera Z, Santana-Rodríguez JJ. Determination of fluoroquinolones in fishes using microwave-assisted extraction combined with ultra-high performance liquid chromatography and fluorescence detection. J. Food Compost Anal. 2017; 56:140-146.
14. Ding Y, Zhang W, Gu C, Xagoraraki I, Li H. Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. J. Chromatogr. A. 2011; 1218 (1): 10–16.
15. Ziarrusta H, Val N, Dominguez H, Mijangos L, Prieto A, Usobiaga A, et.al. Determination of fluoroquinolones in fish tissues, biological fluids, and environmental waters by liquid chromatography tandem mass spectrometry. Anal, Bioanal, Chem. 2017; 409(27): 6359-6370.
16. Li Y, Li Q, Zhou K, Sun X, Zhao L, Zhang Y . Occurrence and distribution of the environmental pollutant antibiotics in Gaoqiao mangrove area, China. Chemosphere. 2016; 147: 25-35. 17. Blesa J, Silva LJ, Lino CM, Font G, Pena A. Comparison of three solid-phase extraction processes in quantification of ciprofloxacin and enrofloxacin in pork meat. J. Sep. Sci. 2012; 35(7): 832–838.
18. Horváth B, Opara-Nadi O, Beese F. A Simple Method for Measuring the Carbonate Content of Soils. Soil Sci. Soc. Am. J. 2005; 69(4): 1066-1068.
19. Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. Environ. Sci. Technol. 2001; 35(17): 3397-406.
20. Van Doorslaer X, Dewulf J, Van Langenhove H, Demeestere K. Fluoroquinolone antibiotics: an emerging class of environmental micropollutants. Sci. Total Environ. 2014; 500-501(1): 250-69.
21. Madej K. Microwave-assisted and cloud-point extraction in determination of drugs and other bioactive compounds. Trends Anal. Chem. 2009; 28(4):436–46.
22. Hu W, Ma L, Guo C, Sha J, Zhu X, Wang Y. Simultaneous extraction and determination of fluoroquinolones, tetracyclines and sulfonamides antibiotics in soils using optimised solid phase extraction chromatography-tandem mass spectrometry. Intern. J. Environ. Anal. Chem. 2012; 92(6):698–713.
23. Leal RM, Figueira RF, Tornisielo VL, Regitano JB. Occurrence and sorption of fluoroquinolones in poultry litters and soils from São Paulo State, Brazil. Sci. Total. Environ. 2012; 15:344-9.

24. Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. Environ. Sci. Technol. 2001; 35(17): 3397- 406.
25. Hernández F, Sancho JV, Ibáñez M, Guerrero C. Antibiotic residue determination in environmental waters by LC-MS. TrAC Trends Anal Chem. 2007; 26(6): 466–85.
26. Kim SC, Carlson K. Quantification of human and veterinary antibiotics in water and sediment using SPE/LC/MS/MS. Anal. Bioanal. Chem. 2007; 387(4):1301-15.
27. Gros M, Rodríguez-Mozaz S, Barceló D. Fast and comprehensive multiresidue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A. 2012; 1248:104–21.
28. Evaggelopoulou EN, Samanidou VF, Michaelidis B, Papadoyannis L. Development and validation of an lc-dad method for the routine analysis of residual quinolones in fish edible tissue and fish feed. application to farmed gilthead sea bream following dietary administration. J. Liq. Chromatogr. Relat. Technol. 2014; 37(15): 2142–2161.
29. Neves MA, Silva GS, Brito NM, Araujo KCM, Marques EP, Silva LK. Aqueous ultrasound-assisted extraction for the determination of fluoroquinolones in mangrove sediment by high-performance liquid chromatography and fluorescence detector. J. Braz. Chem. Soc. 2018; 29(1): 24–32.
30. Azzouz A, Ballesteros E. Combined microwave-assisted extraction and continuous solid-phase extraction prior to gas chromatography–mass spectrometry determination of pharmaceuticals, personal care products and hormones in soils, sediments and sludge. Sci. Total. Environ. 2012; 419: 208–215.
31. Roca M, Castillo M, Marti P, Althaus RL, Molina MP. Effect of heating on the stability of quinolones in milk. J. Agric. Food. Chem. 2010; 58(9):5427–5431.
32. Lillenberg M, Yurchenko S, Kipper K, Herodes K, Pihl V, Sepp V, et.al. Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization-mass spectrometry. J. Chromatogr. A. 2009; 1216(32): 5949–5954.
33. Martı´nez-Carballo E, Gonza´lez-Barreiro C, Scharf S, Gans O. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ. Pollut. 2007; 148(2): 570-579.

التقدير الكمي للفلوروكوينولونات في التربة الملوثة بواسطة كروماتوغرافيا السائل العالي الاداء مع الاستخلاص بالطور الصلب

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الخلاصة:

يهدف البحث الى تطوير طريقة تحليلية كمية لتحليل ثلاثة انواع من الادوية وهي السايبروفلوكساسين، النورفلوكساسين والاوفلوكساسين الموجودة في التربة الملوثة. تم تنفيذ الطريقة المقترحة باستخدام الاستخلاص بمساعدة الميكروويف (MAE) ، الاستخلاص بالطور الصلب (SPE) لغرض تنقية النماذج، ومن ثم تحليل العينات بواسطة جهاز كروماتوغرافيا السائل العالي الاداء- وكاشف الاشعة فوق البنفسجية. في هذه الدراسة تم اختبار عدد من المذيبات العضوية لغرض استخلاص المركبات الدوائية قيد الدراسة، وتم تقييم كفاءة الاستخلاص من خلال اختبار عدد من المتغيرات مثل نوع المذيب، حجم المذيب، وقت الاستخلاص، درجة الحرارة وعدد مرات الاستخلاص. أظهرت طريقة التحليل خطية جيدة بمدى تراكيز $1 - 300\ ng\ g^{-1}$ مع معامل ارتباط $(r^2 > 0.998)$ لكل المركبات المدروسة. كما اظهرت الطريقة الحالية قيم استردادية جيدة تراوحت بين 99% – 89 ، وقيمة انحراف قياسي نسبي اقل من 7%، أما حدود الكشف والكمية فقد تراوحت بين $0.9 - 2.7\ ng\ g^{-1}$. تم تطبيق الطريقة الحالية على نماذج تربة حقيقية اخذت من ناحية جديدة الشط (ديالى – العراق) وقد رويت هذه التربة بماء الصرف الصحي ، وقد أشارت النتائج الى وجود المركبات قيد الدراسة في نماذج التربة بتراكيز تراوحت بين $1.9 - 4.6\ ng\ g^{-1}$. أكدت النتائج المستحصلة على ان الطريقة الحالية ملائمة لتقديرالمركبات الدوائية قيد الدراسة في التربة الملوثة ، كما اشارت الى قابلية المركبات الدوائية على التراكم في التربة التي تتعرض الى مياه الصرف الصحي.

الكلمات المفتاحية: التربة الملوثة، مركبات الفلوروكوينولون، كروماتوغرافيا السائل العالي الاداء، الاستخلاص المساعد بالمايكروويف، الاستخلاص بالطور الصلب.