



Research Article

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Method Validation for Detection of Ciprofloxacin Residues in Hospital's Drinking Water in Khartoum State, Sudan

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Abstract

In fluoroquinolone antibiotic group ciprofloxacin antibiotic is used for the treatment of human and animal infection because of their wide antibacterial spectrum. The rampant and extralable use of these antibiotics could lead to contamination of environment. This study describes a simple salting-out liquid-liquid extraction technique for simultaneously detection of ciprofloxacin residue in 13 drinking water samples from two hospitals (Alacademy & Eltamouz). The high-performance liquid chromatographic ultra violet visible detection (HPLC-UV) containing C18 X-bridge analytical column was used for the determination of this antibiotic in water samples. The range of concentration of CIP in samples was between 3-654 µg/ml. The developed method has linear range of 50-500 µg/ml with the linearity coefficient of 0.9989. The limit of detection and limit of quantification of the method for ciprofloxacin was 61.2 and 185.5 µg/ml respectively. The recoveries for the studied antibiotic ranged from 22.9-151.6% with relative standard deviations between 0.62 and 1.19%. The method has advantages of simplicity, easy operation and consumption of low volume of the less hazardous organic solvent.

Keywords

Ciprofloxacin, HPLC-UV, Limit of detection, Limit of quantification

Introduction

The discovery of antibiotics is considered the greatest scientific and medical milestone of the 20th century [1]. Antibiotics have been detected in various compartments of the aquatic environment, e.g. wastewaters, surface and ground water and in drinking water as well [2]. They are released to the aquatic environment in different pathways. After the administration to humans and animal, they are excreted as metabolites but also a considerable amount is eliminated in unchanged form as parent compounds via urine and faeces into the sewage [3]. Hospitals are also one of the most important contributors of the occurrence of the antibiotics into the aquatic environment [4]. The presence of pharmaceuticals in drinking water, especially antibiotics, even at very low concentrations, should raise reasonable concerns among stakeholders such as drinking-water regulators, governments, water suppliers and the public, with regard to the potential risks to humans [5]. During the last decades, the presence of antibiotics in the environment has aroused an increasingly concern worldwide they are now consider as emerging pollutants. Fluoroquinolone antibiotics (FQs) are derived from quinolones and are widely used in human and veterinary medicine due to their broad activity spectrum against Gram negative bacteria through the inhibition of DNA gyrase and good oral absorption [6]. Ciprofloxacin is

approved for human use and it's a first choice drug to treat gastrointestinal infection. Ciprofloxacin, an antibacterial of the quinolone group, was developed from these molecular changes. It is the antibiotic most active against Gram-negative bacteria of the class and is widely used in urinary and respiratory tract infections, as well as against skin, bone, and joint infections [7]. Various chromatographic detection techniques have been developed for the determination of FQ residues such as HPLC-UV, HPLC-PDA and LC-MS [8]. HPLC methods are widely applied because of their high selectivity, sensitivity and simple sample treatment using different detection systems. As the residues of antibiotics are usually

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present at very low concentrations in the environmental water, a sample preparation and pre-concentration step are necessary before analysis [9]. Several extraction procedures have been previously developed for the pre-concentration of antibiotics from water matrices including solid phase extraction (SPE), liquid-liquid extraction (LLE), and salting-out assisted liquid-liquid extraction for different pharmaceutical drugs [10]. Each of these methods has its own advantages and disadvantages. Salting-out assisted liquid-liquid extraction (SALLE) is based on the phase separation of water-miscible organic solvents from the aqueous solutions in the presence of high concentration of salts. It uses water-miscible organic solvents which generally have low toxicity as the extractants, and the use of salts causes almost no pollution to the environment [11]. It is a cost effective technique and also take less time for sample preparation. Having such benefits, SALLE was selected to extract ciprofloxacin from water sample in the present study. The objective of this study was the optimization of analytical parameters for the extraction by SALLE and determination of ciprofloxacin antibiotic residue in water samples using HPLC-UV.

Materials and Methods

Instruments

High performance liquid chromatography (HPLC KNAUER -B) (Model: AZURA) code No: QC042, PH meter (Model: Metrohm) code No: QC031, Centrifuge (Model: Baird & Tatlock) code No: QC007 & Ultrasonic (Model: Decon) code No: QC004.

Chemicals & reagents

Ciprofloxacin (CIP) (99.93%), analytical standard was purchased from Aarti Drug Limited, Co, India. Analytical grade salt used was ammonium acetate (Sd Fine Chem Limited), Co, India. Hydrochloric acid, acetonitrile & Methanol were purchased from (DUKSAN), South Korea, & Orthophosphoric acid (Scharlau), Turkey.

Method

Preparation of standard solutions: An individual Standard stock solution of Ciprofloxacin (CIP) was prepared at a concentration of 0.5 mg/ml, by dissolving an accurately weighed quantity of each compound in distilled water and adjusting to volume. The standard solutions were stored at -20 °C. Working solutions were prepared daily by appropriate dilution of aliquots of the standard stock solutions in HPLC grade water. The working solutions were used for preparation of calibration curves of concentration 50, 100, 200, 300 and 500 µg/ml.

Sample extraction procedure: CIP residue from water samples was extracted as per the method described by Gezahegn, et al. (2019) with some modification [12]. The 100 ml water sample was first centrifuged at 1000 RPM for 10 min, filtered through 1.25 µm filter and pH was adjusted to 3 using 0.1N HCL solution. Sample was then spiked with a predetermined volume of the standard solution containing the target analyze and quantitatively transferred the 50 ml

Table 1: The concentration of CIP in water samples.

Sample number	Concentration of CIP in µg/ml
A1	109
A2	140
A3	160
A4	4
A5	129
T1	156
T2	627
T3	654
T4	215
T5	467
T6	3
T7	459
T8	221

beaker. Next, 50 ml acetonitrile was added and shaken in ultrasonic device for 5 min, and then 40g CH₃COONH₄ were added to mixture. Thereafter, the solution was shaken again in ultrasonic device for 10 min to ensure complete dissolution of the salt. This was followed by centrifugation of the solution at 1000 rpm for 10 min which resulted in phase separation. The upper organic phase was carefully withdrawn using micro-pipette and the extract was collected in a clean beaker and concentrated to dryness at 60 °C in the oven. The residues were dissolved in 10 ml methanol:water (20:80) and filtered through a 0.22 µm syringe filter and stored at -20 °C for further analysis [13]. An injection volume of 15 µL was finally injected into the HPLC system for detection of the residue in water sample. The concentration of ciprofloxacin in drinking water samples was shown in Table 1.

HPLC analysis: CIP was separated on a Waters X Bridge C18 column (25 cm × 4.6 mm, 5 µm, (Milford, MA, USA) at 40 °C with a flow rate of 1.5 ml min⁻¹. The injection volume was 15 µL and the detection was on UV visible at 278 nm. The mobile phase used for this study was proposed by Moema, et al. [13] which consisted of A (acetonitrile) and B (orthophosphoric acid) at pH 3 [14].

Method validation: The proposed method was validated for different performance criteria; linearity, intraday assay and interday assay, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The linearity response was examined by triplicate analysis of standard solution with CLP at five levels (50, 100, 200, 300 and 500 µg/ml). The standard calibration curves were obtained by plotting concentrations (µg/ml) against peak area. LOD and LOQ were calculated from the standard deviation (σ) of y-intercepts of regression analysis and the slope of calibration curve (m) using equations: $3.3 \sigma/m$ and $10 \sigma/m$ respectively. Also, the LOD repeatability was determined as relative standard deviation (%RSD) to the concentration 200 µg/ml 6 times. The precision of the method consists of intraday assay precision and interday assay precision and expressed as %RSD of peak area measurements. The intraday assay precision was

determined at three concentrations 50, 200 & 300 µg/ml and evaluation was done through the results obtained with the method operating over 2 days under the same conditions. The inter-assay precision was determined by spiked 3 water samples at three fortification levels, 50, 100 and 200 µg/ml and the analyses were performed over the period of three consecutive days. The accuracy of the method expressed as recovery % was determined by triplicate analysis of three fortification levels (50, 100 and 200 µg/ml) [15], and the results were shown in Table 2.

Results and Discussion

Extraction of ciprofloxacin from water

SALLE is an environmental-friendly technique that generally uses organic solvents with low toxicity and salts that almost are not pollutants for the environment. But the development of unique sample preparation in SALLE requires extraction solvent, type of the salt, solution pH. For extraction studies, the hospitals water samples fortified with antibiotic standard solutions (200 µg/L) were employed. Several water-miscible organic solvents such as acetonitrile, acetone, methanol, ethanol, and ethyl acetate can be used in the SALLE. Acetonitrile is the most conventional solvent used in SALLE due to being chemically inert with organic analytes, and its wide use as the mobile phase in liquid chromatography. Various inorganic and organic salts such as sodium chloride (NaCl), magnesium sulfate (MgSO₄), sodium acetate (CH₃COONa), ammonium sulfate ([NH₄]₂SO₄), and ammonium acetate (CH₃COONH₄) can provide the salting-out effect and enhance the transfer of hydrophilic analytes to the organic solvent. It is necessary that the selected salt needs to be soluble in the aqueous sample and have partial solubility in the organic solvent. The pH value is important as it affects the ionization status as well as the solubility of the analytes. For extraction of different pharmaceuticals and antibiotics from water, various extraction solvents and salts described in the literatures such as methanol, acetone, acetonitrile and salts like NaCl, MgSO₄ at solution pH 3, 4 and 7 were evaluated with best results obtained from acetonitrile and ammonium acetate salt at pH 3 that resulted in simultaneous extraction of ciprofloxacin antibiotic from water with good recoveries. The acetonitrile as extracting solvent and (CH₃COONH₄) as extracting salt has been used by many researchers for the extraction of various pharmaceutical including antibiotics from water with good results [16]. The concentration of CIP in Eltamouz hospital is higher than in Alacademy hospital this is due to the unknown health effects of chronic low-level exposure to antibiotics over a lifetime if the antibiotic survives drinking water treatment and is present in consumer's drinking water, improper disposal of expired medicines, landfill leachates and residue from hospital waste. The procedure was applied to the analysis of ciprofloxacin in 13 drinking water samples from two hospitals, analysis showed that all water samples were positive for ciprofloxacin residue these means the large consumption of antibiotic in two hospitals. The range of concentration of CIP in samples is between 3-654 µg/ml, the trace levels of CIP in the water have shown to have great impact on human. Contamination

Table 2: Method validation parameters for HPLC-UV method optimized for the determination of CIP in water sample.

Method Validation Parameters	CIP
Linear range (µg/ml)	50-500
Linearity (R ²)	0.9989
LOD (µg/ml)	61.2
LOQ (µg/ml)	185.5
Accuracy (Recovery %)	
50 µg	22.99%
200 µg	102.6%
300 µg	151.62%
Intraday assay precision, n = 3 (RSD %)	
50 µg	0.836
200 µg	0.998
300 µg	0.244
Inter day assay precision, n = 3 (RSD %)	
50 µg	0.832
200 µg	1.11
300 µg	0.755

has been found to harm aquatic species and over many years, an increase in the levels of contamination may pose greater harm to humans, so to reduce this, drugs should be disposed of in an accessible and protected way in order to stop improper disposal (Table 1).

Method evaluation

Table 2

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Conclusion

A SALLE method coupled with HPLC-UV was presented for extraction, identification and quantification of ciprofloxacin in water samples, and successfully utilized for the determination of CIP residue in environmental water. The above method offered a number of features including good linearity, high recovery, and short analysis time, simple operation process, cost effective, and environmentally friendly.

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