WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 4, 134-142. Research Article ISSN 2277– 7105

STATISTIC ANALYSIS & METHOD VALIDATION FOR DETECTION OF CIPROFLOXACIN RESIDUES IN HOSPITAL'S DRINKING WATER IN KHARTOUM STATE, SUDAN

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Article Received on
08 Jan. 2023,
Revised on 28 Jan. 2023,
Accepted on 18 Feb. 2023
DOI: 10.20959/wjpr20234-27350

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ABSTRACT

This study describes a simple salting-out liquid–liquid extraction technique for simultaneously detection of ciprofloxacin residue in 41drinking water samples from four hospitals (Alacademy & Eltamouz, Ibrahim Malek & Suba). 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 0-928µg/ml. The developed method has linear range of 50-500µg/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 185.5µg/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 observed compatibility and deviations were determined by comparing the results statistically using various statistical programs SPSS PASW-23, and significant differences accepted at $p \leq 0.05$.

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 gastro intestinal 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 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 500µg/ml.

Sample Extraction procedure

CIP residue from water samples was extracted as per the method described by Gezahegn *et al.* (2019)[12] with some modification. 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 beaker. Next, 50 ml acetonitrile was added and shaked in ultrasonic device for 5 min, and then 40 g CH_3COONH_4 were added to mixture. Thereafter, the solution was shaked 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 0.22µm 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 (25cm × 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.* (2012)[14] which consisted of A (acetonitrile) and B (orthophosphoric acid) at pH 3.

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 500µg/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: 3.3σ/m and 10σ/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.

Statistical data analysis

The computer software ‘‘Statistical Program for Social Sciences "(*SPSS, PASW* Statistics *23*, SPSS Inc.) was used for data analysis. The analysis of the experimental data was carried out using *Mann-Whitney* U (2-tailed) and Kruskal –Wallis H tests, the differences were considered significant if calculated *p-values* were <0.05. Pearson product-moment correlation coefficient computational software program in SPSS was also applied to determine the difference between four hospitals in term of concentrations of ciprofloxacin in drinking water samples, and the results were shown in table 3 & figure 1.

RESULTS AND DISCUSSION

Table 1: The concentration of CIP in water samples.

Sample number	Concentration of CIP in µg/ml
A1	109
A2	143
A3	160
A4	4
A5	129
T1	156
T2	627
T3	654
T4	215
T5	467
T6	3
T7	459
T8	221
S1	190
S2	861
S3	838
S4	928
S5	38
S6	1
S7	342
S8	63
S9	230
S10	114
S11	69
S12	191
S13	2
S14	604
S15	3
S16	0
S17	14
S18	0
S19	26
S20	0
I1	40
I2	0
I3	22
I4	29
I5	84
I6	59
I7	75
I8	12

Table 2: Difference between hospital in term of CIP.

Hospital	Sample size	Mean	Std. Deviation	95% Confidence Interval for Mean		F-test	P-value
				Lower Bound	Upper Bound		
Suba	20	225.70	317.661	77.03	374.37	34.513	0.000
Ibrahim malek	8	40.13	30.121	14.94	65.31		
El academy	5	109.00	61.608	32.50	185.50		
El tamouz	8	350.25	235.319	153.52	546.98		
Total	41	199.56	262.969	116.56	282.56		

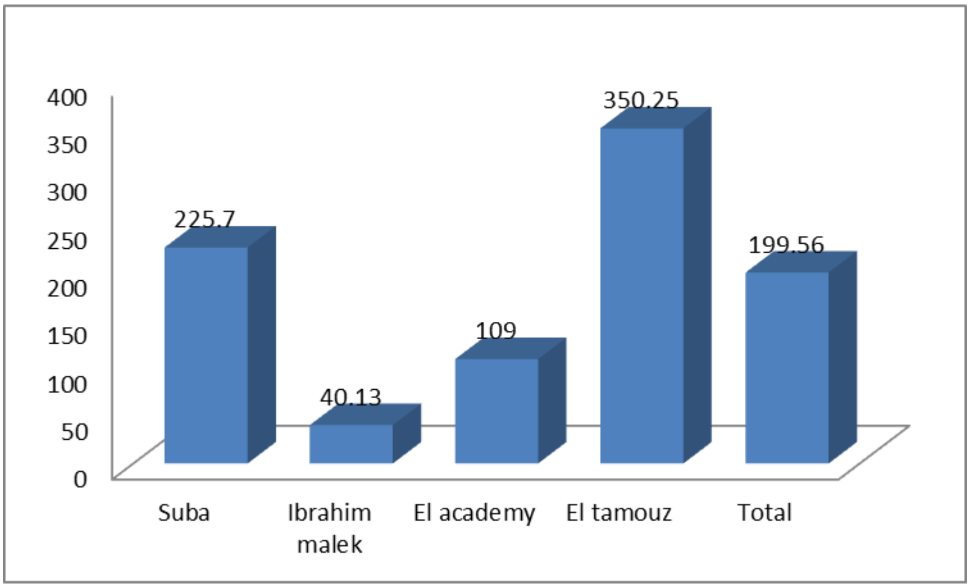

Figure 1: data analysis of ciprofloxacin residues in four hospitals.

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 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, $MgSO_4$ 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 ($CH_3COO\ NH_4$) as extracting salt has been used by many researchers for the extraction of various pharmaceutical including antibiotics from water with good results. From analysis data, the P-value of F-test (0.000) is less than significant level (0.05) that indicates, there is statistical difference between four hospitals in term of CIP supported El tamouz (large mean of CIP in water). The concentration of CIP in Eltamouz hospital is higher than in 4 hospitals 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 0-928µg/ml, the trace levels of CIP in the water have shown to have great impact on human. Contamination 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.

Method Evaluation

Table 3: 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 %) 50µg 200µg 300µg	 22.99% 102.6% 151.62%
Intraday assay precision, n=3 (RSD %) 50µg 200µg 300µg	 0.836 0.998 0.244
Inter day assay precision, n=3 (RSD %) 50µg 200µg 300µg	 0.832 1.11 0.755

ACKNOWLEDGEMENT

The authors would like to thanks to Abdel Moneim Medical Industries (CIMA) for funding the research project.

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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