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Determination of Fluoroquinolone Antibiotics in Industrial Wastewater by High-Pressure Liquid Chromatography and Thin-Layer Chromatography–Densitometric Methods

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Key Words

Ciprofloxacin hydrochloride
Moxifloxacin hydrochloride
High-performance liquid chromatography
Thin-layer chromatography–densitometry
Industrial wastewater

Summary

Two methods were described for the simultaneous determination of ciprofloxacin HCl (CIP) and moxifloxacin HCl (MOX) in their binary mixture present in industrial wastewater. A solid-phase extraction procedure (SPE) based on retention on HLB OASIS cartridges and elution with a mixture of methanol–water in acidic medium was preformed, and then both fluoroquinolones were separated using two chromatographic methods. The first method was based on high-performance liquid chromatographic separation of the two drugs on reversed-phase Zorbax C18 column. The mobile phase consisted of monobasic potassium phosphate (50 mM, pH 2.5, adjusted with phosphoric acid) and acetonitrile (80:20, *v*/*v*). Flow rate was 1 mL min^{-1}. Quantitation was achieved with ultraviolet (UV) detection at 278 nm. Linearity was found to be over the concentration range of 1–50 µg mL^{-1} for both CIP and MOX. The second method was based on the thin-layer chromatographic (TLC) separation of the two drugs followed by densitometric measurements of their bands at 278 nm. The separation was carried out on silica gel 60 F_{254} plates, using methanol, ammonia, and methylene chloride (55:35:20, *v*/*v*) as a developing system. The linearity was found to be in the range of 0.25–2.5 µg band^{-1} for both CIP and MOX. Both methods were optimized and validated as per International Conference on Harmonization (ICH) guidelines. Separation was developed on spiked water samples and checked on process wastewaters of industrial origin after SPE sample pretreatment.

1 Introduction

Pharmaceutical compounds are widespread contaminants of the aquatic environment. Since traditionally they have not been viewed as environmental contaminants, the study of their presence in the environment is in some ways a new area of research which has taken in recent years. Our current knowledge indicates that residues of pharmaceuticals at trace quantities are widely spread in aquatic systems [1]. Antibiotics constitute a large group of pharmaceuticals, which are widely administered in human and veterinary medicine. The extensive use of these antibiotics may result in their presence in the environment. Antibiotics are believed to be of greatest concern among all pharmaceuticals due to the potential risk of antibiotic resistance. Studies in the United States of America and Europe have detected antibiotic resistant bacteria in drinking water supplies [2, 3]. According to previous studies and publications, one of the most prevalent groups of antibiotics found in the environment, and particularly in surface waters, is that of the widely used, highly potent fluoroquinolones (FQs) [4–6]. Administered FQs are largely excreted as unchanged compounds in urine, and consequently discharged into hospital sewage or municipal wastewater. Despite lots of studies with positive detection of antibiotics and other pharmaceuticals in soils and environmental waters and despite of their negative effects on human health, there is no defined limit value for the occurrence of these pollutants in soils or natural waters.

Therefore, more monitoring and surveillance studies are needed at local level to determine exactly how the antibiotics make their way into public waterways and to obtain a better understanding of the transport and environmental fate of antibiotics.

Ciprofloxacin hydrochloride (CIP) [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid] and moxifloxacin hydrochloride (MOX) [1-cyclopropyl-7-[(1*S*,6*S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-quinolone-3-carboxylic acid] are fluoroquinolone antibacterial agents which are active against a wide range of bacteria. Their chemical structures are shown in **Figure 1**.

Different methods are available for the determination of the selected FQs, CIP and MOX in environmental water samples. Water samples are analyzed after solid-phase extraction, by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [7], fluorescence detection (FD) [8, 9], mass [10] spectrometric [11], tandem mass spectrometric detection [12], high-performance liquid chromatography (UPLC) with diode-array detection (DAD) fluorescence and mass spectrome-

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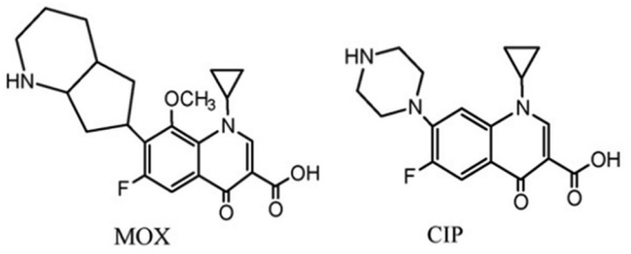

Figure 1

The chemical structures of (a) CIP and (b) MOX.

try detection [13–15], UPLC tandem mass spectrometry [16–18], and capillary electrophoresis [19].

The aim of this work was to develop simple, accurate, and sensitive HPLC and TLC–densitometric methods for the simultaneous determination of the selected FQs, CIP, and MOX in industrial wastewater.

2 Experimental

2.1 Apparatus

2.1.1 For HPLC Method

HPLC Agilent 1200 series, vacuum degasser, thermostated column compartment G1316A/G1316B, multiple wavelength detector, quaternary pump (Germany), and chromatographic column of Zorbax ODS (4.6 cm × 250 mm, 5 µm) were used.

2.1.2 For TLC–Densitometric Method

CAMAG (Muttenz, Switzerland) TLC instrumental set-up consisting of sample applicator Linomat V, 100-µL syringe (Hamilton, Switzerland) and TLC Scanner III operated by winCATS software (V 3.15, CAMAG, Switzerland) were used. Precoated silica gel 60 F_{254} plate (20 × 10 cm^2) (Fluka Chemie, Buchs, Switzerland) with 200 µm thickness was employed. A UV lamp – short wavelength 278 nm was employed for detection of bands.

2.1.3 Solid-Phase Extraction

SPE apparatus consisted of a 12-port vacuum manifold with drying attachment and 12 large volume samplers.

Table 1

Location of wastewater collection areas.

Samples	Location
Industrial wastewater 1, 2	Pharmaceutical company located in Obour city
Industrial wastewater 3, 4	Pharmaceutical company located in 10th of Ramadan city
Industrial wastewater 5	Pharmaceutical company located in El Sawah
Industrial wastewater 6	Pharmaceutical company located in October city

2.2 Material

2.2.1 Samples

Ciprofloxacin HCl and moxifloxacin HCl powders were kindly supplied by the Egyptian Pharmaceutical Industrial Company (EPICO, Egypt) and El-Rowad Company (RIPC, Egypt), respectively, and their percentage purities were found to be 100.1 ± 0.95 and 99.99 ± 0.58, respectively, according to official British Pharmacopoeia (BP) methods [20].

2.2.2 Chemicals

Acetonitrile, methanol, and water of HPLC grade; methylene chloride and ammonia of analytical grade; potassium dihydrogen orthophosphate; and orthophosphoric and ultra-pure sulfuric acids were obtained from Merck (Darmstadt, Germany). Sodium chloride and ethylenediaminetetraacetic acid (EDTA) disodium salt were obtained from El Nasr Company (Cairo, Egypt).

Membrane filters 0.45 µm from Teknokroma (Barcelona, Spain) were used. The cartridges used for SPE were Oasis HLB (6cc 200 mg) (Waters Corp., Milford, MA, USA).

2.3 Standard Solutions

2.3.1 Stock Solutions

CIP and MOX were prepared by dissolving 100 mg of each powder in 100 mL of distilled water, forming a solution with a final concentration (1 mg mL^{-1}).

2.3.2 Working Solutions

Working solutions of both CIP and MOX were freshly prepared by dilution from their stock solutions with mobile phase to obtain a concentration of 100 µg mL^{-1} for CIP and MOX for HPLC method and diluting with distilled water to obtain concentration of 250 µg mL^{-1} for TLC–densitometric method.

2.4 Procedure

2.4.1 Collection and Preparation of Samples

A total of six wastewater samples were collected from pharmaceutical industries at different sites as shown in **Table 1** and placed in 2-L amber glass bottles. Water samples were centrifuged at 4500 rpm for 5 min to remove possible solid material and stored in the dark at 4°C [14, 21]. Fluoroquinolones were concentrated from water samples by solid-phase extraction method.

2.4.2 Extraction and Clean-Up (SPE Procedure)

After adjustment of the pH to 4.0 with sulfuric acid (1 M) and addition of 186 mg of EDTA dipotassium salt, 1 L of the sample was percolated through the Oasis HLB 6 cc (200 mg) cartridge. The cartridge was previously conditioned with 5 mL of methanol and 4 mL of ultrapure water. The washing step was performed with ultrapure water at pH 4.0. Then the cartridge was eluted with 4 mL of methanol. This eluate was evaporated to dryness under a gentle stream of nitrogen and the residue was redissolved in 0.5 mL of mobile phase, and filtered through 0.45 µm Millipore membrane filter (Billerica, MA). The injec-

tion volume of eluate was 20 µL in case of HPLC while dissolved in methanol in case of TLC–densitometry.

Because of the zwitterionic nature of FQs, SPE of their analytes is expected to be strongly pH-dependent. HLB can be used in both acidic and in basic solutions. For these considerations, samples were usually acidified to pH 3 to reduce biological activity, cartridges conditioning and sample loading were done at pH 3 [22–25].

2.4.3 HPLC Method

2.4.3.1 Chromatographic Conditions

Chromatographic separation of the binary mixture was performed using an isocratic elution of a mobile phase consisting of sodium dihydrogen phosphate (50 mM, pH 2.5, adjusted with phosphoric acid) and acetonitrile (80:20, *v*/*v*). The mobile phase was filtered through 0.45-µm membrane filter and degassed for 30 min in an ultrasonic bath prior to its use. The mobile phase was pumped through C18 column at a flow rate of 1 mL min^{-1}. Analyses were performed at ambient temperature, and detection was carried out at 278 nm. The injection volume was 20 µL.

2.4.3.2 System Suitability

Twenty microliters of the working solutions were injected. The system suitability parameters, retention time, tailing factor, theoretical plate count (*N*), height of theoretical plate (HETP), separation of eluted peaks (resolution), and column capacity were calculated and compared to the reference United States Pharmacopeia (USP) guidelines [26].

2.4.3.3 Construction of Calibration Curves

Aliquot volumes (0.1–5 mL) of CIP and MOX working solutions (100 µg mL^{-1}) were transferred separately into 10-mL measuring flasks, diluted to the volume with the mobile phase. Twenty microliters of these solutions were injected in triplicate into the HPLC system. The chromatographic conditions were applied, and the chromatograms were recorded. Calibration curves relating to the peak areas $\times 10^{-3}$ of CIP and MOX *versus* their concentration were plotted, and the corresponding regression equation was calculated.

2.4.4 TLC–Densitometric Method

2.4.4.1 Chromatographic Conditions

TLC aluminum sheets, 20 × 10 cm, precoated with 0.25 mm silica gel 60 F_{254} were used. The samples were applied as bands (band width: 6 mm, bands were spaced 1 cm apart from each other and 1.5 cm from the bottom edge of the plate). Linear ascending development was done in a chromatographic tank previously saturated with methylene chloride–methanol–ammonia (55:35:20, *v*/*v*) for 1 h at room temperature to a distance of approximately 8 cm from the lower edge. The developed plates were air dried and scanned at 278 nm on CAMAG TLC Scanner 3 operated in the absorbance mode, with deuterium lamp as a source of radiation; the slit dimension was kept at 3 mm × 0.45 mm, and 20 mm s^{-1} scanning speed was employed.

2.4.4.2 System Suitability

Parameters including resolution (R_s) and peak symmetry were calculated and compared to the reference USP guidelines [26].

2.4.4.3 Construction of Calibration Curves

Aliquots (1–10 mL) of CIP and MOX working solution (250 µg mL^{-1}) equivalent to 250–2500 µg were accurately transferred into a series of 10-mL volumetric flasks, and the volumes were made up to the mark with distilled water to give final concentrations of 25–250 µg mL^{-1}. Aliquots (10 µL) of each concentration equivalent to 0.2 –2.5 µg were applied to the TLC plates as bands. Calibration curves relating to the peak areas $\times 10^{-3}$ of CIP and MOX *versus* their concentration were plotted, and the corresponding regression equations were calculated.

2.4.5 Assay of Laboratory-Prepared Mixtures

Different aliquots of the drugs were accurately transferred from their working solutions and mixed to prepare solutions of different ratios. The chromatographic conditions of both methods were adopted for each laboratory-prepared mixture, and the concentrations of each drug were calculated from the corresponding regression equation. Each concentration was conducted from the average of three experiments.

2.4.5 Spiked Water Samples

Samples of pure water spiked with different concentration of CIP and MOX were treated with previous SPE procedure, then the adopted chromatographic conditions were applied, and the recovery of each drug was calculated.

3 Results and Discussion

The aim of this work was to develop two simple chromatographic methods for the simultaneous determination of CIP and MOX in industrial wastewater. In both methods, we overcome the problem of the interference of impurities by choosing the proper clean-up procedure and mobile phase.

3.1 HPLC Method

Improved resolution of both components was achieved using a mobile phase composed of sodium dihydrogen phosphate buffer adjusted at pH 2.5 with orthophosphoric acid and acetonitrile (80:20, *v*/*v*).

To optimize the HPLC method, it was necessary to test the effect of different variables. In order to separate the two drugs from each other. Two types of stationary phases were tried (Zorbax C8 and Zorbax SB-C18 columns), but the latter showed a more suitable resolution. Several ratios of buffer solution and acetonitrile were checked. Increasing the ratio of acetonitrile slightly caused some broadening for both peaks. Using methanol instead of acetonitrile was not successful for the separation of CIP and MOX. The pH of the mobile phase is a major factor influencing the chromatographic behavior of FQs; thus, different PHs were examined. Best peak shape and adequate separation of the two drugs were obtained by a final composition of sodium dihydrogen phosphate buffer adjusted at pH 2.5 with orthophosphoric acid and acetonitrile 80:20 (*v*/*v*) as shown in **Figure 2**.

The calibration curves for both drugs were constructed between peak area × 10^{-3} at 278 nm *versus* the corresponding concentra-

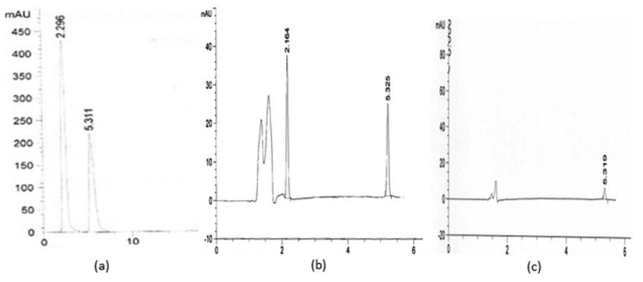

Figure 2

HPLC chromatogram of (a) laboratory-prepared mixture showing the separation of CIP at (t_R = 2.296 min) and MOX at (t_R = 5.311 min) using the applied chromatographic conditions, (b) the determination of CIP and MOX in spiked wastewater after solid-phase extraction using the applied chromatographic conditions, and (c) the determination of MOX in wastewater sample.

tion for both drugs, and linear relationships were obtained in the range of 1–50 μg mL^{-1} and for both CIP and MOX.

The parameters of system suitability of this method were compared to reference values [27]. The results are listed in **Table 2**.

Results obtained by applying the HPLC procedure showed that the method is valid for the simultaneous determination of CIP and MOX in the presence of each other in the laboratory-prepared mixtures with mean percentage recovery of 100.63 ± 0.57 and 100.9 ± 0.5 for CIP and MOX, respectively (**Table 3**).

3.2 TLC–Densitometric Method

The TLC–densitometric method offers a simple way for direct separation on TLC plate, followed by measuring the optical density of the separated bands of CIP and MOX at 278 nm.

Studying the optimum parameters for maximum separation was carried out by trying different developing systems with different ratios, but complete separation of both drugs was achieved by using methanol, ammonia, and methylene chloride (55:35:20, *v*/*v*) as a mobile phase, which gave good resolution, sharp, and symmetrical peaks. Different scanning wavelengths were tried; on using 278 nm, the separated peaks were more sharp and symmetrical with minimum noise. The R_F values were as follows: CIP (0.43 ± 0.02) and MOX (0.54 ± 0.02) as shown in **Figure 3a**.

The parameters of system suitability of the TLC–densitometric method were compared to reference values [26]. The results are listed in Table 2.

The calibration curves were constructed by plotting the integrated peak area ×10^{-3} at 278 nm *versus* the corresponding concentration of both drugs. Linear relationships were obtained in the range of 0.25–2.5 μg band^{-1} for both CIP and MOX.

Results obtained by applying the TLC–densitometric procedure showed that the method was valid for the simultaneous determination of CIP and MOX in the presence of each other in the laboratory-prepared mixtures, with mean percentage recoveries of 100.5 ± 0.64 and100.46 ± 0.73 for CIP and MOX, respectively (Table 3).

3.3 SPE

When an analyte is present at low concentration in complex environmental samples, such as wastewaters, extraction and preconcentration must precede chromatographic analysis. In this work, solid-phase extraction with HLB extraction cartridges was used for sample preparation. The extraction recoveries of the analysts were estimated using spiked water samples. Antibiotics were extracted from water adjusted at pH 4.0. They were eluted from the disks with methanol. The spiked samples were extracted in triplicate and analyzed by HPLC–UV and TLC–densitometry as shown in Figures 2b and 3b, respectively. The recovered amount was calculated as peak area. Extraction recoveries for all investigated antibiotics are given in **Table 4**.

4 Method Validation

Method validation was performed with all the proposed methods as follows:

4.1 Range and Linearity

The linearity of both methods was evaluated by processing a minimum of 6-point calibration curves on three different days.

Table 2

Statistical analysis of parameters required for system suitability of HPLC and TLC–spectrodensitometric methods.

Parameter	RP-HPLC method		TLC–densitometric method		Reference value [25, 27]
	CIP	MOX	CIP	MOX	
t_R (RP-HPLC) R_F (TLC)	2.2	5.32	(0.43 ± 0.02)	(0.54 ± 0.02)	t_R > 1 (HPLC)
N (column efficiency)	7096	13,960			*N* > 2000 Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	0.002	0.001			The smaller the value, the higher the column efficiency
T (tailing factor)	1.03	0.89	1	1	*T* < 2 *T* = 1 for symmetric peak
R_s (experimental resolution)	7.3		1.66		R_s > 1.5

Table 3

Determination of CIP and MOX in laboratory-prepared mixtures by applying the proposed methods.

Mixture No.	Ratio	RP-HPLC method						TLC–densitometric method					
		CIP			MOX			CIP			MOX		
		Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery %	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery %	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery %	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery %
1	1:1	2	2.01	100.54	2	1.99	99.54	0.8	0.803	100.32	0.8	0.798	99.77
2	1:2	2	1.99	99.69	4	4.03	100.69	0.4	0.404	100.98	0.8	0.797	99.65
3	1:3	1	1.01	100.80	3	2.99	99.66	0.2	0.203	101.43	1.6	1.610	100.63
4	1:5	1	1.01	101.18	5	5.00	100.01	0.2	0.201	100.32	1	1.009	100.93
5	2:1	4	4.04	100.93	2	2.01	100.59	1.2	1.197	99.76	0.6	0.608	101.32
Mean ± SD				100.63 ± 0.57			100.9 ± 0.5			100.5 ± 0.64			100.46 ± 0.73

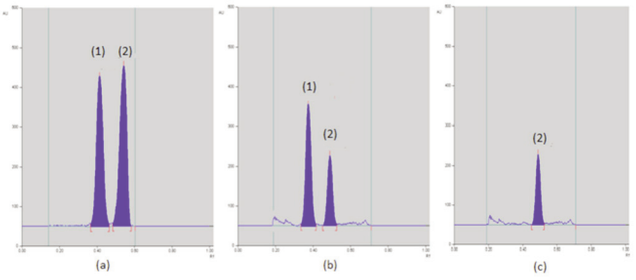

Figure 3

TLC chromatogram of (a) (1) laboratory-prepared mixture of 0. 5 μg band^{-1} of CIP and (2) 0.25 μg band^{-1} MOX using methylene chloride–methanol–ammonia (55:35:20, *v*/*v*) as developing system, (b) spiked wastewater after solid-phase extraction of (1) 0.75 μg band^{-1} of CIP and (2) 2.75 μg band^{-1} MOX, and (c) wastewater sample showing the detection of MOX at R_F value of 0.48.

The corresponding concentration ranges, calibration equations, and other statistical parameters for both methods are listed in **Table 5**.

4.2 Limits of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated, respectively, for both drugs using the proposed methods with a ratio of 3.3 and 10 standard deviations of the blank and the slope of the calibration line (Table 5).

4.3 Accuracy

To study the accuracy of the proposed methods, procedures under study of linearity, for both drugs using the proposed methods, were repeated three times for the determination of five different concentrations of pure CIP and MOX. The accuracy expressed as percentage recoveries is shown in Table 5. Good accuracy of the developed methods was indicated by the results obtained.

4.4 Precision

The precision of the proposed methods, expressed as RSD, was determined by the analysis of three different concentrations of pure CIP and MOX within the linearity range. The intra-day precision was assessed from the results of three replicate analyses of three pure samples CIP and MOX on a single day. The inter-day precision was determined from the same samples analyzed on three consecutive days. The results of intra-day and inter-day precisions are illustrated in Table 5.

4.5 Specificity

Specificity was ascertained by analyzing different mixtures containing both drugs in different ratios as listed in Table 3. The separated drugs in the prepared mixtures were confirmed by comparing their retention times and/or R_F values to those of standard solutions and the increase in peak response after spiking of standards in tested wastewater. Other parameters such as resolution, capacity factor, and selectivity for the separated spots and peaks were calculated.

Table 4

Mean percent recoveries of FQs in spiked water using the proposed chromatographic methods.

Fluoroquinolones	For HPLC method		For TLC–densitometric method	
	Spiked level (mg mL^{-1})	Recovery %	Spiked level (mg mL^{-1})	Recovery %
CIP	2.5 (LOQ)	87.1	0.35 (LOQ)	81.6
	50	92.2	2.5	89.3
MOX	2.5 (LOQ)	89.5	0.3 (LOQ)	79.6
	50	91.1	2.5	91.5

Table 5

Assay parameters and validation sheet for the proposed chromatographic methods.

Parameters	TLC–densitometric method		HPLC method	
	CIP	MOX	CIP	MOX
Wavelength (in nm)	278			
Calibration range[a)]	0.25–2.5	1–50		
LOD[a)]	0.110	0.101	0.849	0.843
LOQ[a)]	0.335(8.375 µg L^{-1})	0.305 (7.625 µg L^{-1})	2.572	2.555
Slope	0.1314	0.092	0.0973	0. 0.016
Intercept	0.2588	0.1786	0.0013	0.0866
Mean %[b)]	100.45	99.89	98.87	100.34
% RSD	1.737	1.413	1.209	1.658
Accuracy[c)]	99.02/0.72	100.60/0.75	100.73/0.97	101.2/0.80
Intra-day precision[c)]	100.08/0.70	100.25/0.65	100.57/0.61	100.2/0.39
Inter-day precision[c)]	100.04/0.64	100.12/0.23	100.21/0.46	100.2/0.46
Robustness[c)]	100.64/0.63	99.63/1.45	99.47/0.93	99.75/1.41
Correlation coefficient (*r*)	0.9995	0.9996	0.9999	0.9999

[a)]RP-HPLC methods: in (µg mL^{-1}); TLC–spectrodensitometric methods: in (µg $band^{-1}$)

[b)]Average of three experiments

[c)]Mean value/relative standard deviations (RSD) of three samples

Table 6

Occurrence of pharmaceuticals in industrial wastewater samples by HPLC and TLC–densitometric methods (mg L^{-1}).

	HPLC method		TLC–densitometric method	
	CIP	MOX	CIP	MOX
W.W1	N.D.	N.D.	N.D.	N.D.
W.W2	N.D	11.25	N.D.	11.3 (0.452 µg $band^{-1}$)
W.W3	1.82	N.D.	1.8 (0.0728 µg $band^{-1}$)	N.D.
W.W4	4.13	N.D.	3.92 (0.156 µg $band^{-1}$)	N.D.
W.W5	N.D.	N.D.	N.D.	N.D.
W.W6	N.D.	N.D.	N.D.	N.D.

N.D. = not detected

4.6 Robustness

For HPLC method, the robustness was investigated by the analysis of samples under a variety of experimental conditions, such as small changes in the pH (3.0–3.5) of the buffer in the mobile phase. The effect on retention time and peak parameters was studied. It was found that the method was robust when the mobile phase ratio was varied. During these investigations, the retention times were modified; however, the areas and peaks symmetry were conserved.

For TLC–densitometric method, the robustness was investigated by the analysis of samples under a variety of experimental conditions, such as small changes in proportions of different compo-

nents by up to ±0.5% mainly of the organic parts of the mobile phase. The effect on R_F values and peak parameters was studied. It was found that the method was robust when the mobile phase ratio was varied. During these investigations, the R_F values and peak symmetry were of minor change; however, the areas were conserved. The effects of robustness are shown in Table 5.

5 Application of Method

The developed and validated analytical method was applied to the determination of the target drugs in wastewater samples from different pharmaceutical industries; a total of six samples of industrial wastewater were analyzed under the previous conditions described.

Thousand milliliters of prefiltered and acidified (with sulfuric acid (1 M), pH 4) wastewater samples were applied to Oasis HLB cartridges. Analytes were eluted with 4 mL of methanol. This eluate was evaporated to dryness under a gentle stream of nitrogen, and the residue was redissolved in 0.5 mL of mobile phase in case of HPLC and methanol in case of TLC–densitometric method. Chromatograms of industrial wastewater samples are shown in Figures 2c and 3c.

The procedures also were applied for industrial wastewater after spiking with known concentration of both standards to check the presence of either drug in concentration less than their LOQs.

Identification of target compounds was done by comparison of TR, R_F values of pharmaceutical standards, and compounds in wastewater samples; the results are stated in **Table 6**.

6 Conclusion

CIP and MOX were extracted and preconcentrated from spiked water samples by solid-phase extraction (SPE) and quantitatively determined by the two proposed methods. This procedure resulted in good recoveries for the antibiotics used in this study.

Both proposed methods provided simple, accurate, and reproducible quantitative analysis of both CIP and MOX in laboratory-prepared mixtures and wastewater samples. The HPLC method was more rapid and gave better resolution between both drugs, thus lowering analysis time and providing high sensitivity and selectivity.

Also the TLC–densitometric method had the advantage over the reported method of using a simpler developing system and was applied for the quantitative estimation of the cited drugs [28].

The method was found to fulfil the validation requirements of the analytical methodology for the determination of CIP and MOX in industrial wastewater.

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