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Validated Chromatographic Methods for Determination of Ciprofloxacin, Indomethacin, and Metronidazole Remnants in Pharmaceutical Industrial Wastewater Effluents

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Abstract

Two simple, rapid, low-cost, and provident chromatographic approaches were introduced and certified for the quantitative estimation of ciprofloxacin, indomethacin, and metronidazole residues in production wastewater samples. Preparation of the samples was made using a solid-phase extraction technique, and before analysis, it was carried out on bond Elut C18 packs. The first technique was TLC densitometric determination at 278 nm. The separation was carried out on TLC (Thin Layer Chromatography) plates (silica gel 60 F254) as a stationary phase and ethyl acetate: methanol: dichloromethane: n-hexane: ammonia (33%) (3.6: 3: 6: 2: 1, by volume) as a mobile phase. The second method was high-performance liquid chromatography (HPLC). The separation has been accomplished on Equisil BDS C18 column and UV (Ultra Violet) detection at 278 nm. Acetonitrile/phosphate buffer mixture of pH 3 (75:25; v/v) is the mobile phase, the pH was adjusted using o-phosphoric acid, which is adapted to a flow rate of 1.5 mL/min. The previously stated techniques have been confirmed according to ICH guidelines. The methods described were used to accurately assess the drug residues studied in laboratory-prepared mixtures and actual industrial waste-water samples to confirm that it is free from these drug residues so it can be recycled and used for irrigation and other purposes.

Keywords: Ciprofloxacin; Indomethacin; Metronidazole; Wastewater; SPE; HPLC; TLC.

1. Introduction

Pharmaceutical deposits Analysis in the aquatic system was a promising research area. These pharmaceutical residues are recurrently discharged to the environment through industrial routes, metabolic excretions, or improper disposal [1]. This incomplete removal of such residues from wastewater increases the chance of contamination of plants and animals with the drug residues, which may help in increasing the risk of antimicrobial resistance. To some extent, it may be toxic to animals and plants. Three of the most commonly used medications were chosen for analysis in industrial wastewater samples in this investigation. Namely Ciprofloxacin (CIP), Metronidazole (MET), and Indomethacin (IND) which are formulated in the factory on the same day each on its line of manufacturing, as CIP was formulated as tablet (Cipro®), MET was formulated as suspension (Metrozole®), and IND was formulated as topical gel (Indacin®). On the end of the day of manufacturing the machinery system was washed and the effluents were directed to the wastewater treatment plants then to the sewage system. The combination of SPE technique with the selected analytical techniques used in this study were able to obtain a good recovery of the selected drugs that indicate no interference from excipients and additives which were confirmed by the results of spiked wastewater samples. Ciprofloxacin

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(CIP) is an antimicrobial agent, chemically is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (Fig. 1a), one of the quinolone antibiotics, with activity against both gm.-negative and gm.-positive microorganisms and other several bacteria, including mycobacteria, rickettsias, mycoplasmas, and protozoa [2]. Metronidazole (MET) 2-methyl-5-nitroimidazole-1-ethanol (Fig. 1b) is used as an antiprotozoal, antibacterial, and anti-amebic drug. Indomethacin (IND) belongs to the class of a Non-steroidal anti-inflammatory drugs (NSAIDs) and is used for the treatment of acute pain of ankylosing spondylitis, acute gouty arthritis, and osteoarthritis. The anti-inflammatory, antipyretic, and analgesic effects of indomethacin are due to the ability to inhibit prostaglandin biosynthesis [3]. Chemically indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-ylacetic acid (Fig. 1c). The absorption spectrum of each drug have been supplied in supplementary file 1.

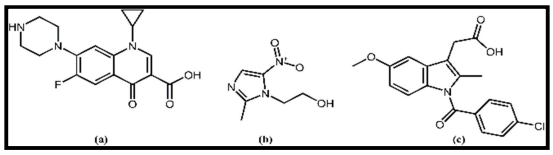

Fig. 1: Chemical structures of (a) CIP, (b) MET, and (c) IND

For the determination of CIP in biological and pharmaceutical samples, several analytical approaches have been reported. These methods include spectrophotometric determination [4]–[7], spectrofluorimetry [8]–[10], HPLC [11]–[15], capillary electrophoresis [16], [17] and HPTLC[18]. CIP, ampicillin, and MET admixture were determined by NMR [19]. MET was analyzed by spectrophotometry [20]–[22] and HPLC [23]–[28]. CIP in intravenous admixture with MET was determined by first-derivative spectrophotometry [29] and LC [30]. Other analytical methods have been designated for the simultaneous determination of CIP, MET. By RP-HPLC and TLC densitometry [31]. Other approaches for determining IND in its pure form and combination with its degradation products have been published. However, no former approaches have been used to determine CIP, MET, and IND in environmental samples simultaneously. The current study uses reversed-phase High-performance liquid chromatography (HPLC) and HPTLC-densitometry to provide two novel methodologies for assessing CIP, MET, and IND in environmental materials. HPLC is often regarded as the best technology for simultaneous measurement of pharmaceuticals contained in multi-component mixtures due to its speed and robustness[32]. It is also simple, accurate, precise, efficient and sensitive. [33]. The presence of lipophilic moieties in the mobile phase increases the retaining of counter charged molecules in the reversed-phase chromatography [34]. In addition, the development of HPTLC-densitometric procedures in respect of practical realization, high flow, and routine feasibility was also followed to afford a quality control (QC) procedure for CIP, MET, and IND in environmental water relaying to an attractive, alternative, competitive, and quantitative approach to HPLC. The two methods advocated are convenient, delicate, reproducible, and fast. They have been intended to be suitable for the quality evaluation of environmental water residues. The choice of these three drugs were based on the fact that they are manufactured in the factory at the same time each on its line, and thus we ensure that the waste include combination of them. On the other hand, two antimicrobial agents have been selected as they may play a significant role in spreading antimicrobial resistance.

Pharmaceutical residues may also occur in levels ranging from traces to ppb, but they pose a substantial problem due to their extensive use, buildups, and biological activity [35]. Recent review papers have analyzed several characteristics of environmental pharmaceutical ecotoxicity [36], [37]. Therefore, innovative, selective, and delicate analytical methods are needed to quantify these residues in various aquatic samples, principally in industrial wastewater [38]. The data derived from these methods can aid in improving wastewater treatment processes in plants (WWTPs) to prevent the discharge into the environment of such undesirable contaminants. In addition, the analysis of actual drug concentrations in aquatic systems could contribute to environmental safety evaluations [39]. Because of the remarked toxicity and hazardous biotic effects of these pharmaceuticals, numerous analytical methods for the quantitation of antibiotics in water samples have been developed. The methods are based mainly on liquid-chromatography-mass spectrometry tandem [40], [41] or gas chromatography coupled with a mass-spectrometry detector [42], [43]. Although these methods are highly sensitive and selective, tedious and time-consuming procedures are required. In addition to the costly solvents and sophisticated devices. HPLC followed by UV/FL detection has been used in residual analysis to a petty degree [44], [45]. The

advantage of TLC-densitometry, as opposed to other chromatographic methods, is that it is simple and fast, as well as cheap [46]. Furthermost of the offered approachesneed sample preparation, isolation, and concentration of objective analytes from multipart matrices before analysis. The disadvantage of the large volumes of solvents are traditionally extracted, such as liquid-liquid extraction, and extremely low in selectivity. Effective alternatives are used, sorbent trapping as solid-phase microextraction (SPME) and solid-phase extraction (SPE) [47]. This study was mainly aimed at developing validated, and cost-effective analytical methods. The first is RP-HPLC with UV detection [48]. TLC-densitometry is the other approach [49]. These precise methods can be intended for concurrent evaluation and repetitive quality control of the drugs studied in industrial wastewater. Target analytes were extracted and pre-concentrated using SPE technique [50].

2. Experimental

2.1. Instruments

•TLC aluminum plate (silica gel 60 F254 (EMD Millipore, Sigma Aldrich), (20*10 cm, 0.20 mm) Hamilton 100 µLmicrosyringe (Germany) Camag Scanner 5 automatic applicator (Switzerland), and TLC Scanner 3 is running using software from WINCATS (Camag, Switzerland).

•UV lamp (Desega - Germany).

•Separation Chromatographic tank (25 × 25 × 9 cm).

•Agilent HPLC system model 1200 (USA), with a chromatographic column of Equisil BDS C18 column.

•Agilent Bond Elut C18 cartridges (USA), mounted on SPE equipment.

•Heidolph rotary evaporator Germany.

2.2. Materials

2.2.1. standards:

In cooperation with MEMPHIS pharmaceuticals and chemical industries (Cairo, Egypt) they kindly provided pure CIP, IND, and MET, which were verified to contain (100.05 percent), (99.97 percent), and (100.13 percent) correspondingly. Their purity was determined by the supplier's certificates of analysis.

2.2.2. Chemicals and reagents:

•Methanol and Acetonitrile HPLC were acquired from Sigma Aldrich (Germany)

•Phosphate buffer pH 3 is freshly prepared

•Extra pure grade Water was gotten from Merk (Germany).

• Ammonia solution (33%), Ethyl acetate, and n-hexane have been bought from EL-NASR chemicals (Egypt).

•The supplementary reagents are of high critical purity.

2.3. Standard Solutions

Each drug's stock solution has been ready by liquefying 100 mg of the medicine in 100 mL of HPLC grade methanol to produce a 0.001 g/mL concentration. The working standard solution for each drug was freshly obtained by diluting it with methanol to 100 µg/mL from its stock solution.

2.4. Samples collection and storage

Five industrial wastewater samples collected from the effluents of the factory before discharging it into the sewage system, and the 5 samples were poised in brown glass bottles. To remove suspended matter, samples have been filtered immediately before extraction through 0.45-µm nylon membrane filters. Each sample was filtered to a volume of about 200 ml. As previously recommended [51], To avoid degradation or depletion, the models were kept at 4°C and shielded from light.

2.5. Procedures

2.5.1. Solid-phase extraction (SPE) procedure (sample preparation)

The SPE procedure was employed to serve as a method for extracting the analytes from the sampling matrices and preconcentrating the analytes prior to their analysis. Fundamental changes have been added to the manuscript to clarify the point. Bond Elut C18 cartridges were used in the SPE technique. Before use, the units were habituated with 4 mL acidified water (pH 2) and 7 mL methanol. The cartridge was filled with a sample volume of 100 mL, and a flow rate of 3 mL/min was maintained. The units were cleaned twice with 4 ml of acidified water (pH 2) after loading the samples to eliminate undissolved and polar compounds. With the assistance of a vacuum, the cartridges may be dried for around 30 minutes after washing to eradicate extra water entirely. The retained remedies have been eluted with 10 mL methanol from the cartridges. The filtrate was dried on a rotary evaporator at 40 ◦ c after elution, and the remnants were dissolved in 1 mL methanol, yielding a 100-fold pre-concentration.

2.5.2. HPTLC method

2.5.2.1. Chromatographic conditions

Camag Linomat 5 automatic 100 µL Hamilton microsyringe was used as an applicator to apply the samples on a TLC plate coated with silica gel 60 F254 (20*10 cm, 0.20 mm). It has been adjusted to 6 mm of bandwidth. Using the mobile phase, the chromatographic compartment was pre-saturated for 10 minutes. The mobile phase composition was ethyl acetate: methanol: dichloromethane: n-hexane: ammonia 33% (3.6:3:6:2:1, by volume), the separation was produced in ascending technique for approx. 8 cm. The plates have been air-dried and scanned with Camag TLC scanner 3 at 278 nm. In absorbance mode, using a deuterium lamp as a radiation source, this TLC scanner was operated. The slit dimension was maintained at 3 mm x 0.45 mm, with a scan rate of 20 mm/s. and all the processes were carried out at ambient temperature

2.5.2.2. Calibration curves development

To produce the corresponding dilutions in the range of 0.4–2.8 µg/band for every drug (CIP, IND, and MET), aliquots (0.4, 0.8, 1.2, 1.6, 2, 2.4, 2,8 µL) of each working standard have been carefully applied on TLC plates. The peak areas of the stated drugs at 278 nm were plotted against their concentrations to create calibration curves. The obtained Regression Equalities were used to conclude the amount of each medication along with the overall study.

2.5.3. HPLC method

2.5.3.1. conditions

With the help of an isocratic elution of a solvent system consisting of acetonitrile: phosphate buffer pH three adapted with o-phosphoric acid (75:25, v/v) with a flow rate of 1.5 mL/min. The pKa values were 3 for MET, 5.56 for CIP, and 8.7 for IND. An effective chromatographic Column Equisil BDS C18 (250*4.6 mm, particle size 5 m) was employed. The injection volume was 20 µL. UV detection was carried out at 278 nm.

2.5.3.2. calibration curves Construction

CIP, IND, and MET liqueurs were transferred exactly into a sequence of 10 ml measuring flasks of each working standard (100µg/ml). To acquire a range of 0.5 – 15 µg/mL each flask was filled to the mark with methanol. These solutions have been loaded into the HPLC system with an injection volume of (20 µL) and measured for three successive replicates. Then the chromatograms were generated. The peak areas of the tested drugs at 278 nm and their corresponding concentrations were utilized to produce calibration curves in contrast to their concentrations. The regression equations were used to estimate the concentration of each medication during the experiment.

2.5.4. Analysis of laboratory prepared mixtures (selectivity)

Five mixes were obtained by mixing and diluting various Aliquots from the working standards to obtain mixtures containing variant ratios of CIP, IND, and MET in the concentration series of (0.4 - 2.8) µg/band for TLC-densitometry and 0.5-12 µg/ml for HPLC), have been analyzed for assessment of selectivity of the method being proposed. The estimated regression equation was used for the calculation of corresponding concentrations.

2.5.5. Extraction efficiency evaluation

For assessment of the efficiency of extraction of the proposed solid-phase extraction procedure. A serial dilution was prepared from CIP, IND, and MET working standard solution (100 µg/mL). By accurately transferring different aliquots into 100 mL measuring flasks to obtain three different concentrations. The samples have been processed using the SPE technique. The resulting extracts were examined using the two recommended procedures (HPLC and TLC), and the appropriate recession equations were utilized in order tocompute the amount of each medicament from the middling of three measures.

2.5.6. Evaluation of environmental waste-water samples

The suggested SPE technique has been used to purify and pre-concentrate five real samples from 100 ml to 1 ml. The earlier procedures have been applied, and from the equivalent recessionequalities, the amount of each substance was determined.

2.5.7. Evaluation of real samples spiked with standard drugs

Each real sample was spiked with a definite conc. of the standard drugs (5µg/mL), then apply the proposed SPE technique; The earlier procedures have been applied, and from the matching regression equations, the concentration of each substance was determined. After subtraction of the added standard

concentration (5µg/mL). The actual concentration of each drug has been calculated.

3. Results and discussion

The objective of this study was to establish verified, environmentally safe, and economical methods for analysis. These precise techniques are ideal for simultaneous estimation of CIP, IND, and MET remnants in pharmaceutical industrial wastewater and periodic quality monitoring. We have overcome the analysis problems of the pharmaceuticals used in complicated aquatic environments, such as wastewater, by correctly selecting a successful extraction / pre-concentration approach and efficiently optimizing the chromatographic methods provided for different contaminants. These quick and simple approaches can enhance the process of treatment in wastewater treatment plants (WWTPs) to prevent the discharge into the aquatic environment of these undesirable chemical residues (pollutants).

3.1. TLC-densitometry method

Several systems with different ratios have been studied to optimize the chromatographic conditions for maximal separation. ethyl acetate: methanol: dichloromethane: n-hexane: ammonia 33% (3.6:3:6:2:1 by volume) as a solvent system achieved the best resolution, symmetrical and crisp peaks for absolute separation of the examined pharmaceuticals as shown in the 3D densitogram in fig. 2. The wavelength for scanning was 278 nm. The Retention volume (Rf) values were as following: CIP (0.17 ± 0.01), IND (0.35 ± 0.02) and MET (0.58 ± 0.03), as shown in Fig. 3(a).

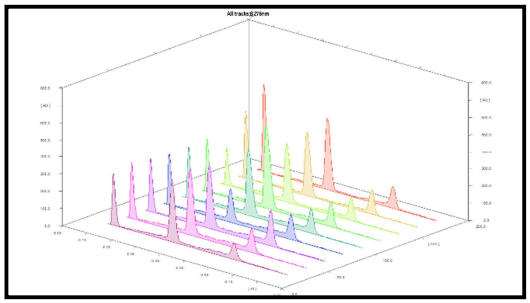

Fig. 2: 3D-TLC densitogram showing linearity range (0.4-2.8 µg/band) of (a) CIP, (b) MET, and (c) IND by using ethyl acetate: methanol: dichloromethane: n-hexane: ammonia (3.6:3:6:2:1, by volume) as a developing system & scanned at 278 nm

3.2. HPLC-UV method

The chromatographic parameters have been tuned in order to get the most appropriate separation method for the considered medications. The complete separation of the studied drugs was obtained by using acetonitrile: phosphate buffer pH three (75:25 V/V) as mobile phase, which attains sharp, symmetrical peaks and the perfect resolution. The separation time was 8 min, and the flow rate was 1.5 ml/min. The UV detection has been set to be 278 nm for the same causes in the TLC method. The retention times (tR) were found to be 2.796± 0.1, 3.489 ± 0.2, and 5.562 ± 0.3 min for MET, CIP, and IND, respectively, as shown in Fig. 3(b).

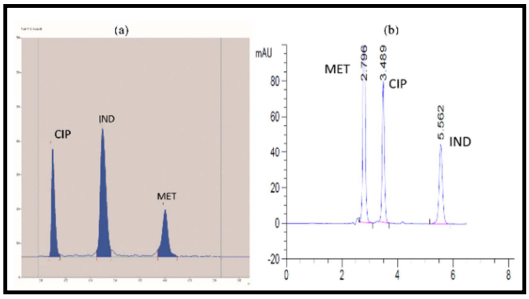

Fig. 3: (a) TLC densitogram, showing CIP at Rf = 0.17, IND at Rf = 0.35, and MET at Rf = 0.58. (b) HPLC chromatogram, showing MET at tR = 2.7 min, CIP at tR = 3.5 min, and IND at tR = 5.5 min.

3.3. Method validation

The suggested methods were validated according to ICH guidelines [52]:

3.3.1. linearity

By charting peak region at 278 nm against respective concentrations in µg/ml, which is equivalent to ppm as it is the universally accepted unit in water analysis for HPLC, and µg/band for TLC procedures, respectively, Calibration graphs for the studied drugs have been constructed. In the range of (0.5-12 µg/mL(ppm); HPLC) and (0.4-2.8 µg/band; TLC), the calibration graphs were linear.

Table I displays linearity, intercepts, slopes, range, and coefficients of determination (R^2) for the methods under investigation. The significance of correlation coefficients showed respectable linearity of the standardization curves.

3.3.2. limit of detection and limit of quantitation (LOD & LOQ)

Table I presents the estimated LOD and LOQ values. The lowest readings show a high sensitivity to the strategies being suggested.

3.3.3. Accuracy and precision

Table I provides % Recovery and % RSD readings that support the techniques' acceptable accuracy and high precision.

3.3.3. System suitability

Both TLC and HPLC techniques' system suitability characteristics were compared to United State Pharmacopeia (USP) reference values. [53]. The results are recorded in Table II.

3.3.4. Robustness

The system's robustness was confirmed by screening samples under a diverse range of experimental situations, such as modest variations in the solvent system ratios of up to 0.5 %. The Rf & tR values have been tweaked somewhat, but the top areas and symmetry have been retained. When the mobile phase ratio was altered, the suggested approaches were found to be robust.

3.4. Application

3.4.1. Analysis of laboratory prepared mixtures (selectivity)

Five lab-prepared mixes containing the drugs in varied proportions were analyzed to measure the chromatographic techniques' selectivity. The separated drugs were verified by comparing Rf & tR values to those of reference solutions. Table III shows the % Recovery of each drug in each prepared mixture.

Table I

Regression and Validation data of the proposed methods

Parameters	TLC-densitometry			HPLC-UV		
	CIP	**MET**	**IND**	**CIP**	**MET**	**IND**
Linearity range [a]	0.4 -2.8			0.5-12		
Slope	2563.4	1351.3	2537.9	44.2	97.538	37.137
Intercept	1256.1	977.57	1631.4	0.8939	3.4447	0.1993
Correlation coefficient (r^2)	0.9998	0.9999	0.9998	0.9999	0.9999	0.9998
LOD [a]	2.16×10^{-2}	1.09×10^{-2}	9.2×10^{-3}	6.8×10^{-3}	1.04×10^{-2}	2.43×10^{-3}
LOQ [a]	6.56×10^{-2}	3.3×10^{-2}	2.8×10^{-2}	2.06×10^{-2}	3.15×10^{-2}	7.37×10^{-3}
Accuracy [b]	100.09 % ± 0.17	100.44 % ± 0.74	99.99 % ± 0.73	100.02 ±0.82	99.84 ± 0.38	100.18 ± 0.42
repeatability [c]	0.302	0.414	0.163	0.489	0.223	0.755
intermediate precision[d]	0.653	0.653	0.562	0.928	0.394	0.797

[a] TLC-densitometry method: in µg/band; HPLC-UV methods: in µg/ml(ppm). [b] Mean ± standard deviation of 3 concentrations of each drug. [c] the repeatability relative standard deviation (% RSD), an average of three various concentrations analyzed three times within the day. [d] the intermediate precision relative standard deviation (% RSD), an average of three various concentrations measured three times in three different days

Table II

System suitability characteristics for the recommended HPLC and TLC-densitometry methods

Parameters	HPLC			TLC-densitometry		
	CIP	MET	IND	CIP	MET	IND
Retention time (tR) (min)	3.49 ± 0.21	2.79 ± 0.11	5.56 ± 0.32	-----	-----	-----
Rf value	-----	-----	-----	0.17 ±0.01	0.58 ± 0.03	0.35 ±0.02
Resolution (Rs) a	9.27	-----	28.48	5.98	-----	4.56

Tailing factor (T)	1.01	1.02	1.09	1.02	1.28	1.01
Retention factor (K)	2.17	1.54	4.05	-----	-----	-----
Selectivity (α) a	1.41	-----	2.63	2.11	-----	2.42
Number of theoretical plates (N)	37886	20665	36532	-----	-----	-----
HETP = Height Equivalent Theoretical Plate (mm)	6.01 x 10-3	12.02 x 10-3	6.82 x 10-3	-----	-----	-----

[a]The parameters were calculated using MET as a reference.

Table III

Evaluation of laboratory prepared mixtures by applying the offered chromatographic methods (Selectivity)

TLC-densitometry				HPLC-UV			
Mixtures ratio	CIP	MET	IND	Mixtures ratio	CIP	MET	IND
(C: M: I)	% Recovery [a]			(C: M: I)	% Recovery [a]		
1:3:6	100.62	99.91	100.13	1:2:1	99.55	100.43	99.86
2:1:4	99.73	99.73	99.92	3:1:4	99.63	100.60	100.15
3:4:1	98.55	99.87	99.49	2:5:1	100.00	99.70	100.09
4:6:5	97.51	99.89	99.89	4:3:2	99.91	99.87	99.99
2:5:3	98.88	100.18	100.42	2:4:3	101.07	100.01	100.18
Mean ± S.D [b]	99.06% ± 1.18	99.91 % ± 0.16	99.96 % ± 0.341	Mean ± S.D [b]	100.03 % ± 0.611	100.12 % ± 0.379	100.05 % ± 0.127

[a]Average of three experiments. [b] Selectivity.

3.4.2. Assessment of Extraction Efficiency

The efficiency of the extraction technique for the SPE process has been measured using lab-spiked water samples at three concentration levels per drug. For the samples prepared, the SPE procedure was applied, and the described methods have been used. The recovery percentage for each medication has been computed, and the extraction recoveries for the pharmaceuticals under consideration are shown in Table IV.

3.4.3. Application to real industrial wastewater samples

The suggested techniques were used to quantify objectivemedicaments in 5 industrial waste-water samples before they were discharged into the sewage system. The HPLC chromatograms of the five samples are available in Fig. 4.

It exhibited a different proportion of the tested drugs. The achieved results are given in Table V.

3.4.4. Verification of objective analytes by standard addition

The approach was used to validate that the resolved chromatographic peaks indicated precisely the respective drugs tested after spiking with a given concentration of standards. As provided in Table VI.

3.6 Statistical analysis and comparison

Table VII shows a statistical comparison of the results produced using the suggested approaches versus the stated CIP and MET methods. It also includes a statistical comparison of the suggested technique to the official USP procedure for IND.

Table IV

The suggested approaches resulted in mean % recoveries of the examined pharmaceuticals in laboratory manufactured spiked water samples

Method	CIP		MET		IND	
	Spiked levels [a]	% Recoveries	Spiked levels [a]	% Recoveries	Spiked levels [a]	% Recoveries
TLC-densitometry	0.5	99.42 ± 0.502	0.5	100.66 ± 1.17	0.5	100.09 ± 0.49

following SPE.

HPLC-UV	1	100.39 ± 1.12	1	100.34 ± 0.75	1	99.49 ± 0.89
	2	99.94 ± 0.23	2	99.93 ± 0.33	2	100.07 ± 0.16
	Mean [b]	99.91	Mean [b]	100.31	Mean [b]	99.89
	1	100.03 ± 0.05	1	100.03 ± 0.04	1	100.42 ± 1.27
	3	99.92 ± 0.23	3	100.16 ± 0.20	3	100.11 ± 0.31
	7	100.03 ± 0.18	7	99.91 ± 0.09	7	100.13 ± 0.20
	Mean [b]	99.99	Mean [b]	100.03	Mean [b]	100.21

[a] HPLC-UV method: in µg/ml (ppm); TLC-densitometry method: in µg/band
[b] Extraction Efficiency (%)

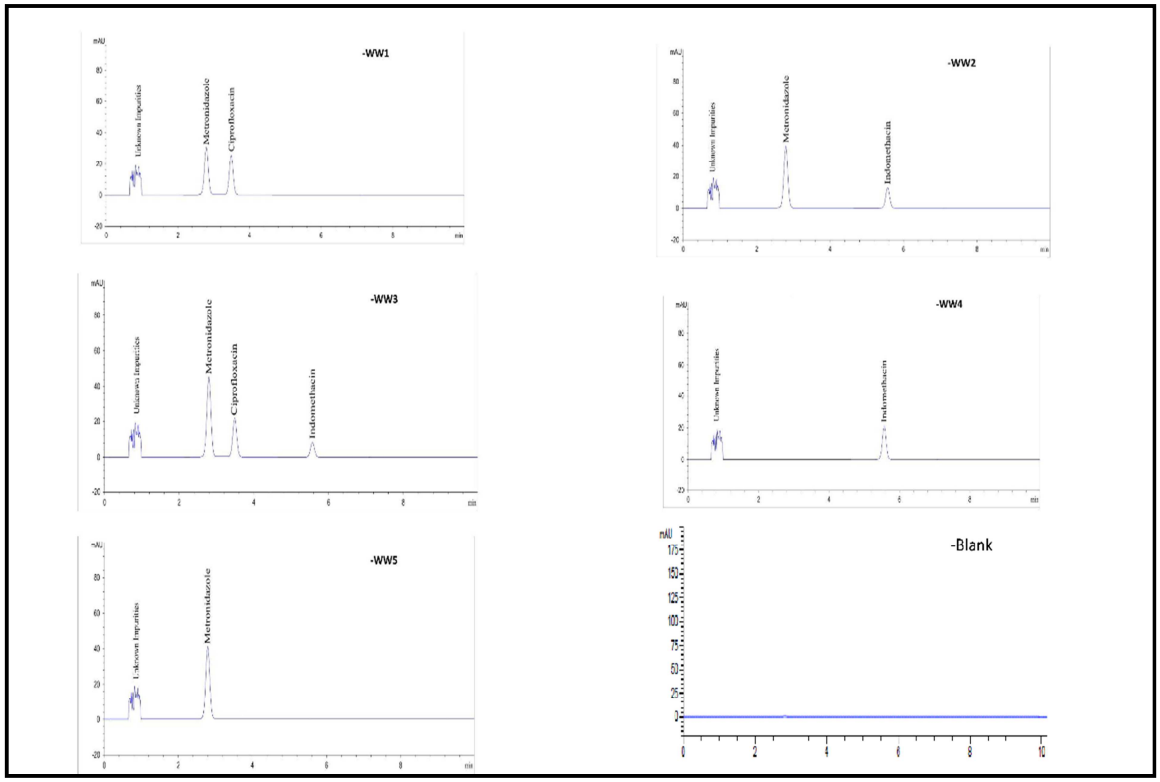

Fig. 4: HPLC chromatograms of blank and the five wastewater industrial samples

Table V

Determination of the studied drugs in industrial wastewater samples by applying the proposed methods

Samples [a]	TLC-densitometry			HPLC-UV		
	CIP	MET	IND	CIP	MET	IND
W.W 1	6.74	3.62	----	6.73	3.68	----
W.W 2	----	4.59	4.13	----	4.61	4.13
W.W 3	5.83	5.43	2.69	5.78	5.43	2.65
W.W 4	----	----	6.61	----	----	6.63
W.W 5	----	4.86	----	----	4.88	----

[a] Samples are calculated in µg/ml (ppm)

Table VI
Analysis of spiked industrial wastewater samples (spiked with 5 µg/mL of studied drugs standards) by using the proposed methods

Samples [a]	TLC-densitometry						HPLC-UV					
	CIP		MET		IND		CIP		MET		IND	
	T.C [b]	D.R [c]	T.C [b]	D.R [c]	T.C [b]	D.R [c]	T.C [b]	D.R [c]	T.C [b]	D.R [c]	T.C [b]	D.R [c]
W.W 1	11.74		8.59		5.01	----	11.74	6.74	8.72	3.72	4.899	----
W.W 2	5.04		9.58		9.13	4.13	5.008	----	9.64	4.64	9.14	4.14
W.W 3	10.83		10.4		7.71	2.71	10.81	5.81	10.46	5.46	7.66	2.66
W.W 4	5.07		4.998		11.59	6.59	5.01	----	5	----	11.64	6.64
W.W 5	4.99		9.86		4.988	----	4.99	----	9.91	4.91	5.011	----

[a] Samples are calculated in µg/ml (ppm). [b] Total drug concentration of the spiked sample in µg/ml (ppm)
[c] Drug residue in µg/ml (ppm) after the subtraction of the added standard

Table VII

Statistical comparison of the findings obtained using the recommended procedures with the official USP methods or published methods

	CIP				MET				IND		
	TLC	Reported TLC method[31].	HPLC	Reported HPLC method[31]	TLC	Reported TLC method[31]	HPLC	Reported HPLC method[31]	TLC	HPLC	Official method[52][a]
Mean	100.09	99.59	100.02	99.85	100.44	99.71	99.84	99.62	99.99	100.18	99.68
± SD	0.17	0.728	0.822	0.188	0.737	0.827	0.380	0.330	0.728	0.42	0.458
RSD %	0.17	0.732	0.822	0.188	0.735	0.840	0.380	0.342	0.728	0.42	0.459
Variance	0.0289	0.5299	0.6724	0.0353	0.5432	0.6839	0.1444	0.1089	0.5299	0.1764	0.2098
N	7	6	7	6	7	6	7	6	7	7	5
Student's t-test	1.644 (2.201)[b]		0.532 (1.79)[b]		1.668 (2.26)[b]		1.117 (2.26)[b]		1.117 (2.26)[b]	1.117 (2.26)[b]	
F value	0.5453 (4.95)[b]		0.52564 (4.95)[b]		1.259 (6.26)[b]		1.3259 (6.26)[b]		1.3259 (6.26)[b]	1.3259 (6.26)[b]	

[a]Official USP method was HPLC for IND. [b] Figures in parenthesis reflect the equivalent tabulated t and F values at P=0.05

The computed t and F values have been lower than the tabulated values, suggesting that there was no significant difference between the offered, official[54], and published procedures[31], demonstrating the suggested methods' excellent accuracy and precision.

Table VIII shows a comparison of the results of LOD, LOQ and the detection type produced using the suggested approaches versus the stated CIP and MET methods. It also includes a statistical comparison of the suggested technique to the official USP procedure for IND.

Table VIII

Comparison of the findings obtained using the recommended procedures with the official USP methods or published methods

CIP	MET	IND

	TLC	Reported TLC method[31].	HPLC	Reported HPLC method[31]	TLC	Reported TLC method[31]	HPLC	Reported HPLC method[31]	TLC	HPLC	Official method[52] [a]
Linearity range [a]	0.4 - 2.8	0.6 -3.2	0.5 - 12	0.8 - 10.2	0.4 -2.8	0.6 -3.2	0.5-12	0.8 - 10.2	0.4 - 2.8	0.5 - 12	0.6 - 10
LOD [a]	2.16×10^{-2}	3.97×10^{-2}	6.8×10^{-3}	1.8×10^{-2}	1.09×10^{-2}	3.12×10^{-2}	1.04×10^{-2}	2.84×10^{-2}	9.2×10^{-3}	2.43×10^{-3}	3.58×10^{-3}
LOQ [a]	6.56×10^{-2}	8.64×10^{-2}	2.06×10^{-2}	4.12×10^{-2}	3.3×10^{-2}	6.82×10^{-2}	3.15×10^{-2}	5.02×10^{-2}	2.8×10^{-2}	7.37×10^{-3}	9.64×10^{-3}
Detection	Densitometric at 278 nm	Densitometric at 280 nm	UV at 278 nm	UV at 280 nm	Densitometric at 278 nm	Densitometric at 280 nm	UV at 278 nm	UV at 280 nm	Densitometric at 278 nm	UV at 278 nm	UV at 254 nm

[a] TLC-densitometry method: in µg/band; HPLC-UV methods: in µg/ml(ppm)

4. Conclusion

Chromatographic methods after SPE-procedure were recommended for the synchronized analyzes of the considered drugs residuals in pharmaceutical manufacturing wastewater. The examined drugs were identified using Rf & tR values and peak regions. The SPE technique provides sufficient recoveries, and the procedures provided have been validated to be a selective, sensitive, environmentally safe, and financial alternative to other urbane approaches.

5. Conflicts of Interest:

The authors declare no conflict of interest.

6. References

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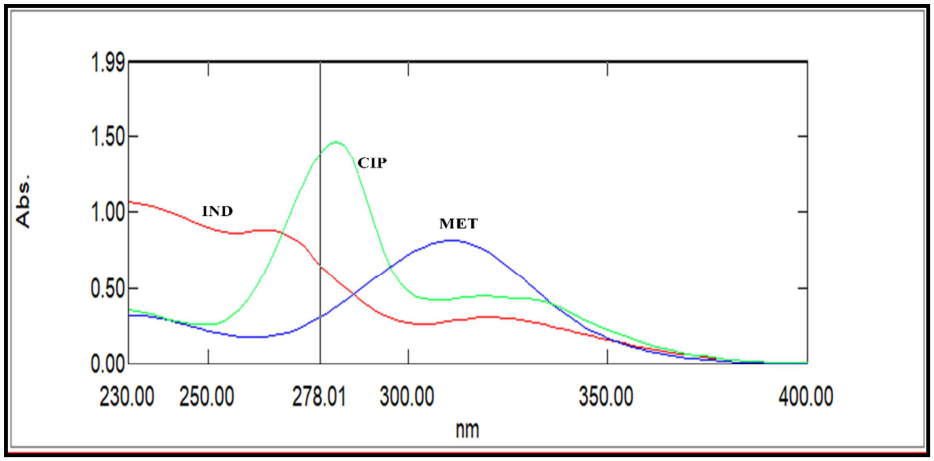
Supplementary file 1



Graphical abstract