GREEN CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF CIPROFLOXACIN, METRONIDAZOLE, AND INDOMETHACIN RESIDUES IN PHARMACEUTICAL INDUSTERIAL WASTEWATER EFFLUENTS

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ABSTRACT

Development and validation of three simple, eco-friendly, accurate and precise chemometric models have been presented for the spectrophotometric determination of ciprofloxacin (CIP), indomethacin (IND), and metronidazole (MET) residues in production wastewater samples. These methods are classical least square (CLS), principal component regression (PCR) and partial least square (PLS-1). A 3-factor 5-level experimental design was built leading to 25 mixtures containing different ratios of CIP, MET, and IND. Thirteen mixtures were used as a training set, and the other twelve were used as a validation set. Using of multi-wavelengths instead of the single wavelength spectrophotometry has greatly improved the precision and predictive abilities of these multivariate calibrations. The proposed methods have been found to be accurate, precise and can be used for determination of the drugs in pure form and industrial wastewater samples without preliminary separation steps. The methods described were used to accurately assess the drug residues in laboratory-prepared mixtures and actual industrial wastewater 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; Chemometrics; Wastewater.

Introduction

Pharmaceutical deposits analysis in the aquatic environment was a promising research area. These compounds are recurrently discharged to the aquatic environment mostly through industrial routes, metabolic excretions, or improper disposal (**Hernando et al. 2006**). This incomplete removal of such residues from wastewater increase the chance of contamination of plants and animals with the drug residues which may increase the risk of antimicrobial resistance and to some extent it may be toxic to

animals and plants. On this study, three of the most widely used drugs have been analyzed in industrial wastewater samples. These drugs are ciprofloxacin (CIP), indomethacin (IND), and metronidazole (MET). Ciprofloxacin, (CIP) is an antimicrobial agent, chemically CIP known as 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (**Figure 1a**). It is one of the quinolone antimicrobial agents, with activity against both gram.-negative and gram.-positive microorganisms and other several bacteria, including mycobacteria, rickettsias, mycoplasmas, and protozoa (**Wilson et al. 2004**). Metronidazole, (MET) 2-methyl-5-nitroimidazole-1-ethanol (**Figure 1b**), is used as an antiprotozoal, antibacterial, and anti-amebic drug. Indomethacin (IND) belongs to the class of heteroarylacetic acid derivatives of 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 (**Roche et al. 2019**). Chemically indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-ylacetic acid (**Figure 1c**).

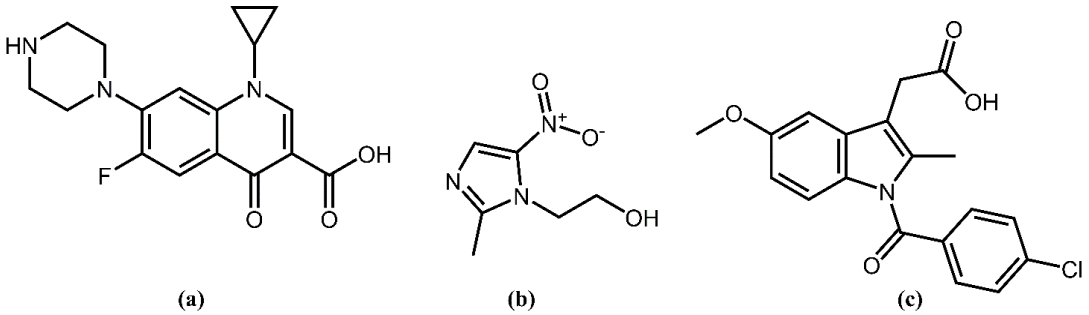

Figure 1: Chemical structures of (a) CIP, (b) MET, and (c) IND

For the determination of CIP in biological and pharmaceutical samples, several analytical approaches have been reported. These methods include spectrophotometric determination (**Fratini and Schapoval 1996**; **Mostafa, El-sadek, and Awad 2002**; **Nagaralli, Seetharamappa, and Melwanki 2002**; **Pascual-Reguera, Parras, and Díaz 2004**), spectrofluorimetry (**Veiopoulou, Ioannou, and Lianidou 1997**; **Navalón et al. 2000**; **Tong et al. 2010**), HPLC (**Thoppil and Amin 2000**; **Maya et al. 2001**; **Zotou and Miltiadou 2002**; **Imre et al. 2003**; **Vybíralová et al. 2005**), capillary electrophoresis (**Bannefeld, Stass, and Blaschke 1997**; **Michalska, Pajchel, and Tyski 2004**) and HPTLC(**Novakovic et al. 2001**). CIP, ampicillin, and MET admixture were confirmed by NMR (**Reinscheid 2006**). MET was analyzed by spectrophotometry (**Nagaraja et al. 2002**; **Saffaj et al. 2006**; **El-Ghobashy and Abo-Talib 2010**) and HPLC (**Galmier et al. 1998**; **Akay et al. 2002**; **Bempong et al. 2005**; **Mishal and Sober 2005**; **Tavakoli et al. 2007**; **Sagan et al. 2005**). CIP in intravenous admixture with MET were determined by first-derivative spectrophotometry (**Vega and Sola 2001**) and LC (**Vega et al. 1999**). Other analytical methods have been designated for the simultaneous determination of CIP and MET by RP-HPLC and TLC densitometry (**Elkady and Mahrouse 2011**). Other approaches for determining IND in its pure form and in combination with its degradation products have been published including spectrophotometric methods(**Maheshwari et al. 2011**; **Jain et al. 2017**; **Ali et al. 2015**; **Rathod et al. 2018**), HPLC methods(**Elbashir and Aboul-enein 2017**; **Sarhangzadeh, Mmohamma-Rezaei, and Jabbri 2014**; **Abed et al. 2020**; **Sataraddi et al. 2014**;

Carbon and Liquid 2020; **Petković et al. 2020**). However, no former approaches have been used to determine CIP, MET, and IND in environmental samples at the same time. Most of the offered methods require sample preparation, isolation, and concentration of target analytes from complex matrices before analysis. The disadvantage of the large volumes of solvents such as liquid-liquid extraction, and extremely low in selectivity. Effective alternatives are based on sorbet trapping such as solid-phase micro-extraction (SPME) and solid-phase extraction (SPE) (**Buszewski and Szultka 2012**). The current study aims to develop and validate simple, sensitive, selective, environmentally friendly, and cost effective chemometric methods namely Classical Least Squares (CLS), principal component regression (PCR), and partial least squares(PLS) for the simultaneous assessment of CIP, MET, and IND in environmental wastewater effluents without preliminary separation. These precise methods can be intended for concurrent evaluation and repetitive quality control of the studied drugs in industrial wastewater. Target analytes were extracted and pre-concentrated by means of the solid-phase (SPE) technique (**Sattar et al. 2021**).

Experimental

Instruments

Shimadzu®UV-Vis. 1800 Spectrophotometer, (Japan) equipped with 10 mm matched quartz cells, UV Probe 2.43 software. Agilent Bond Elut C18 cartridges (USA), mounted on SPE equipment, pH meter (Jenway®, 3510, USA).

Software

UV-Probe personal spectroscopy software version2.1. (SHIMADZU). All chemometric methods were implemented in Matlab R2013b (8.2.0.701), using PLS toolbox software version 2.1. The *t*-test and *F* test were performed using Microsoft_ Excel.

Materials

Pure standard

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 %), (99.97 %), and (100.13 %) correspondingly.

Industrial wastewater samples

Five industrial wastewater samples were collected in amber glass bottles from various manufacturing areas.

Reagents and solvents

Methanol HPLC grade was obtained from Sigma Aldrich (Germany), Hydrochloric acid, (Piochem® Co., Egypt), prepared as 1M aqueous solution.

Standard Solutions

Each drug's stock solution has been prepared by dissolving 100 mg of the drug in 50 mL of HPLC grade methanol and the volume was completed to the mark with the same solvent to produce a 1 mg/mL concentration. The working standard solution for each drug was freshly obtained by diluting it with methanol to a concentration of 100 µg/mL from its stock solution.

Spiked water samples

Five water samples were spiked with different concentrations of the studied drugs as shown in table 1.

Table 1: spiking level of the five water samples

Sample NO.	CIP (µg/mL)	MET (µg/mL)	IND (µg/mL)
1	4	1	3
2	2	5	4
3	3	2	2
4	5	3	1
5	1	4	5

Samples collection and storage

Five industrial wastewater samples from various manufacturing areas were collected in amber 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 (**Turiel, Bordin, and Rodríguez 2005**), To avoid degradation or depletion, the samples were kept at 4°C and shielded from light.

Procedures

Experimental design for chemometric methods

A 5-level, 3-factor design was performed using five concentration levels for the tested drugs. The

design spans the mixture space fairly well; where there are 5 mixtures for each compound at each concentration level, resulting in 25 mixtures. The central level of the design is 3 µg/mL for each drug. **Table 2** represents the concentration design matrix. The absorption spectra of the prepared 25 mixtures were recorded over the wavelength range 220-400 nm with 1 nm interval. Thus the

produced spectral data matrix has 25 rows representing different samples and 181 columns representing wavelengths (25 x 181). Thirteen mixtures of this design were used as a calibration set and the other 12 mixtures were used as a validation set to test the predictive ability of the developed multivariate models.

Solid phase extraction (SPE) procedure

Bond Elut C18 cartridges were used in the SPE technique. Before using, the cartridges were conditioned with 7 mL methanol and 4 mL acidified water (pH 2). The cartridge was filled with a sample volume of 100 mL and a flow rate of 3 mL/min was

maintained. The cartridges 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 entirely eradicate extra water. The retained remedies have been eluted with 10 mL methanol from the cartridges.

Analysis of spiked water samples by the proposed methods

The prepared lab mixed samples were analyzed and the spectra of these solutions were scanned from 200 to 400 nm and analyzed by the proposed methods.

Analysis of industrial wastewater samples by the proposed methods

Suitable dilutions were made using methanol to prepare aliquots from the purified water samples. The spectra of these solutions were scanned from 200 to 400 nm and analyzed by the proposed methods.

Table (2): Experimental design of concentrations of CIP, MET, and IND mixtures used in chemometric methods:

No. of Mix	CIP (µg/ml)	MET (µg/ml)	IND (µg/ml)
1	3	3	3
2*	3	1	1
3	1	1	5
4	1	5	2
5	5	2	5
6	2	5	3
7	5	3	2
8	3	2	2
9	2	2	4
10	2	4	5
11	4	5	4
12	5	4	3
13	4	3	5
14	3	5	5
15	5	5	1
16	5	1	4
17	1	4	1
18	4	1	3
19	1	3	4
20	3	4	4
21	4	4	2
22	4	2	1
23	2	1	2
24	1	2	3
25	2	3	1

* The shaded rows represent the validation set.

Results and discussion

The zero-order absorption spectra of CIP, MET, and IND shows severe overlapping, as shown in **Figure 2**. The spectral overlapping of the drugs prevents resolution of the mixture by the direct spectrophotometric measurements. Thus; we developed an accurate and simple chemometric methods for determination of CIP, MET, and IND in their admixtures and in industrial wastewater samples. The first step in model building, involves constructing the calibration matrix for CIP, MET, and IND. In this study the model was optimized with the aid of the 5-level 3-factor design(**Brereton 1997**) resulting in 25 sample mixture. These 25 sample mixtures were divided to 13 training mixtures (odd numbers) for building the models and 12 validation mixtures (even numbers) for measuring predictive power of the models. The quality of multi component determination depends on the wavelength range and spectral mode used (**Espinosa-Mansilla, Durán-Merás, and Salinas 1998**). The wavelengths used were in the range of 220-400 nm. Wavelengths less than 220 nm were rejected due to the noisy content. Wavelengths more than 400 nm were not used because they were uninformative (no absorption is observed in these regions). Cross-validation methods leaving out one sample at a time was employed (**Hubert et al. 2007**). The predicted concentrations were compared with the known concentrations of the compounds in each calibration sample. The root means squares error of cross-validation (RMSECV) was calculated for each method for examining the errors in the

predicted concentrations. The selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with additional factor. three factors were found to be optimum for the mixture of CIP, MET, and IND using PCR (**Figure 3**) and for PLS-1 there were also three factors (**Figure 4**). The percentage recoveries of the validation samples are shown in **Table 3** indicated the high predictive abilities of PCR, PLS and CLS models. The results obtained by applying the proposed methods compared to those obtained by applying the reported method for CIP and MET(**Elkady and Mahrouse 2011**) and Official USP method for IND and no significant difference was observed (**Table 5**) for CIP and MET and (**Table 6**) for IND.

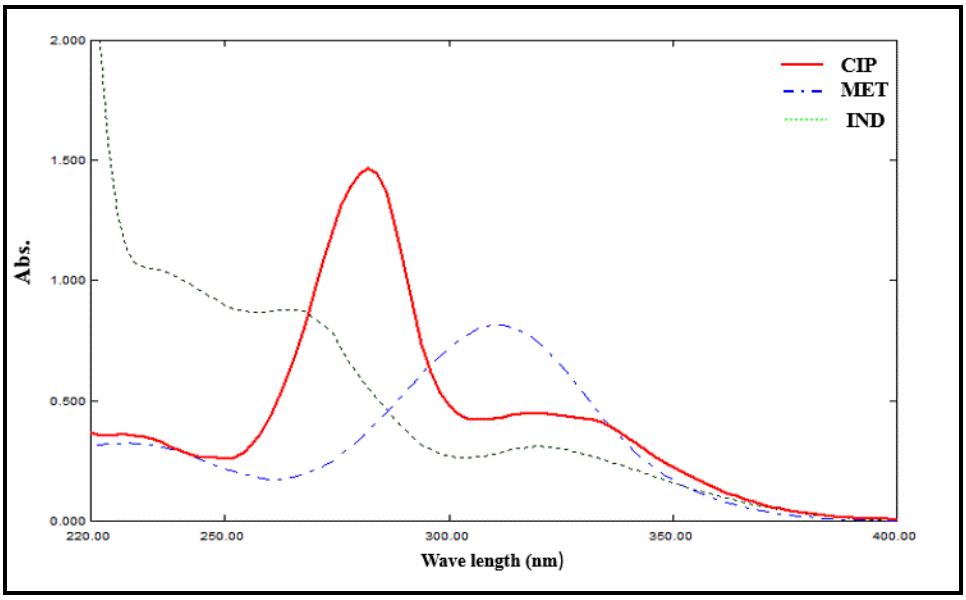

Figure (2): zero order absorption spectra of CIP, MET, and IND (10 µg/mL) for each drug

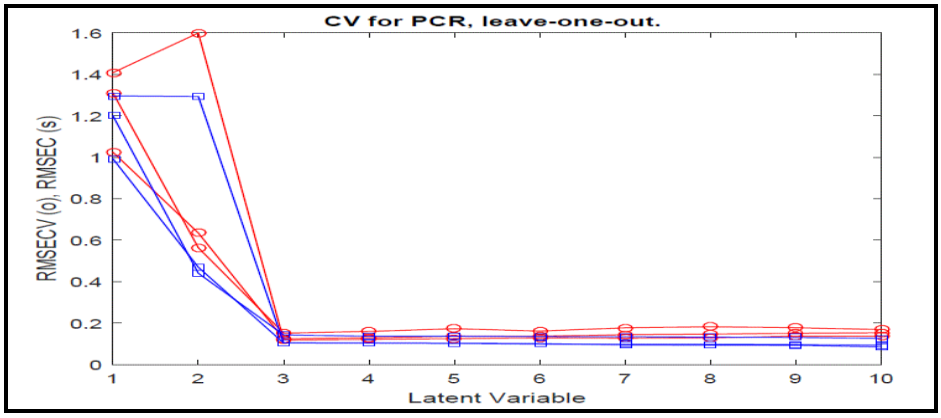

Figure (3): RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PCR model.

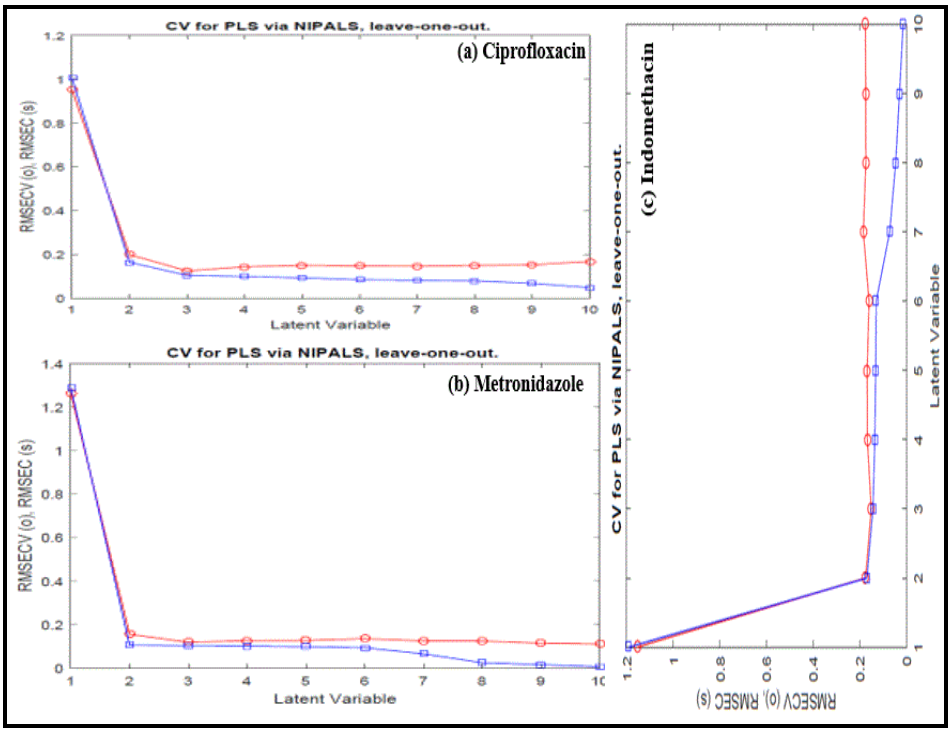

Figure (4): RMSECV plot of the cross validation results of the calibration set as a function of the number of latent variables (LVs) used to construct the PLS model for (a) CIP, (B) MET and (c) IND.

Table 3: Determination of CIP, MET and IND in validation set by the proposed chemometric methods.

Validation mixture*	CLS[a]			PCR[a]			PLS[a]		
	CIP	MET	IND	CIP	MET	IND	CIP	MET	IND
2	95.62	100.88	101.02	100.87	101.78	101.36	98.70	100.76	101.66
4	98.40	97.87	101.90	100.59	99.81	100.74	100.65	97.61	101.40
6	100.83	99.75	100.80	101.41	98.00	100.34	102.63	99.94	100.34
8	103.16	98.64	100.55	100.34	98.65	100.56	100.48	96.87	105.12
10	101.70	97.45	96.09	100.22	98.75	99.50	103.22	99.02	99.87
12	99.07	98.23	100.48	96.88	99.48	100.85	98.92	99.54	99.28
14	99.16	99.89	99.96	99.38	98.80	99.59	99.42	99.51	97.64
16	99.66	99.39	99.58	98.10	101.47	97.69	99.50	100.29	99.69
18	100.22	101.69	100.39	100.36	100.42	99.19	100.28	101.70	98.07
20	99.61	102.27	102.11	99.58	100.58	100.77	96.63	102.13	98.23
22	99.18	100.95	98.41	98.86	100.88	96.26	98.84	100.79	102.05
24	102.00	101.95	99.21	101.71	100.67	98.63	101.21	101.54	101.40
Mean±	99.88±	99.91±	100.04±	99.86±	99.94±	99.62±	100.04±	99.97±	100.39±
%RSD	1.95	1.65	1.62	1.39	1.21	1.50	1.79	1.59	2.08
RMSEP**	0.0522	0.0565	0.0639	0.0563	0.0397	0.0359	0.0457	0.0496	0.0554

* Mixture number related to experimental design.

** Root mean square error of prediction.

[a] the values supplied are recovery values.

Application to industrial wastewater samples:

Five purified wastewater samples have been collected and prepared with the proposed SPE technique. The spectra of these solutions were scanned from 200 to 400 nm and analyzed by the proposed methods. The concentrations obtained are given in **table 4**.

Table 4: Concentration of the studied drugs in wastewater samples (W.W 1 - W.W 5).

Sample NO.	CIP (µg/mL)	MET (µg/mL)	IND (µg/mL)
W.W 1	6.73	3.68	----
W.W 2	----	4.61	4.13
W.W 3	5.78	5.43	2.65
W.W 4	----	----	6.63
W.W 5	----	4.88	----

Table 5: Statistical comparison for the results obtained the proposed methods and reported method(Elkady and Mahrouse 2011) for the analysis of CIP and MET in spiked water samples.

Parameters	CIP			MET			Reported Method****
	CLS	PCR	PLS	CLS	PCR	PLS	
n*	5	5	5	5	5	5	5
$\bar{X}$**	99.93	100.32	100.15	99.62	100.73	99.65	99.66
SD	0.254	0.732	0.488	0.696	0.825	0.612	0.495
Variance	0.065	0.536	0.238	0.484	0.681	0.375	0.307
Student's-t-test***	0.943 (2.36)	1.485 (2.36)	1.390 (2.36)	0.099 (2.36)	2.220 (2.36)	0.036 (2.36)	———
F-value***	4.723 (6.388)	1.834 (6.388)	1.289 (6.388)	1.576 (6.388)	2.218 (6.388)	1.221 (6.388)	———

***** Number of experiments.

** The mean of percent recovery of spiked water samples.

*** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)

**** the reported method was HPLC method for simultaneous determination of both CIP and MET

Table 6: Statistical comparison for the results obtained the proposed methods and Official USP method("USP 2021 Pdf (United State Pharmacopeia 44 - NF 39) - Pharmaceuticals Industry - Web of Pharma" n.d.) **for the analysis of IND in spiked water samples.**

Parameters	CLS	PCR	PLS	Official USP Method****
n*	5	5	5	5
$\bar{X}$**	100.20	100.11	100.54	99.51
SD	0.536	0.732	0.853	0.502
Variance	0.287	0.536	0.727	0.315
Student's-t-test***	1.902 (2.36)	1.368 (2.36)	2.085 (2.36)	———
F-value***	1.097 (6.388)	1.701 (6.388)	2.307 (6.388)	———

***** Number of experiments.
** The mean of percent recovery of drugs spiked water samples.
*** The values in parenthesis are tabulated values of "t "and "F" at (P = 0.05)
******** Official USP method ("USP 2021 Pdf (United State Pharmacopeia 44 - NF 39) - Pharmaceuticals Industry - Web of Pharma" n.d.)

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طرق خضراء لقياس الطيف الضوئي بمساعدة الكيميائيات لتقدير رواسب سيبروفلوكساسين ومترونيدازول وإندوميثاسين في تيارات مياه الصرف الصناعية الصيدلانية

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تم تطوير والتحقق من ثلاثة نماذج قياس كيميائي بسيطة وصديقة للبيئة ودقيقة لتحديد بقايا سيبروفلوكساسين وميترونيدازول وإندوميثاسين في عينات مياه الصرف الصحي لخطوط الإنتاج. هذه الطرق هي المربعات الصغرى الكلاسيكية وانحدار المكون الرئيسي والمربع الجزئي الصغرى. تم بناء تصميم تجريبي مكون من ٥ مستويات من ٣ عوامل يؤدي إلى ٢٥ مزيجًا يحتوي على نسب مختلفة من المستحضرات المذكورة. تم استخدام ثلاثة عشر مزيجًا كمجموعة تدريب ، واستخدمت الاثني عشر الأخرى كمجموعة للتحقق من الصحة. أدى استخدام الأطوال الموجية المتعددة بدلاً من القياس الطيفي ذو الطول الموجي الفردي إلى تحسين الدقة والقدرات التنبؤية لهذه المعايرات متعددة المتغيرات بشكل كبير. تبين أن الطرق المقترحة دقيقة ويمكن استخدامها لتحديد هذه الأدوية في صورها النقية وعينات مياه الصرف الصناعي دون خطوات فصل أولية. تم استخدام الطرق الموصوفة لتقييم بقايا الأدوية التي تمت دراستها بدقة في الخلائط المعدة في المختبر وعينات مياه الصرف الصناعي الفعلية للتأكد من خلوها من مخلفات الأدوية بحيث يمكن إعادة تدويرها واستخدامها للري ولأغراض أخرى.

الكلمات المفتاحية: سيبروفلوكساسين؛ ميترونيدازول؛ إندوميثاسين؛ مياه الصرف الصحي؛ القياسات الكيميائية.