BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e18691

Article

Solid Phase Extraction and Simultaneous Chromatographic Quantification of some Non-steroidal Anti-inflammatory Drug Residues; an Application in Pharmaceutical Industrial Wastewater Effluent

Mohamed A. R. Korashy[1*], Sherif A. Abdel Gawad[2,3], Nagiba Y. Hassan[3], M. AbdelKawy[4]

[1] Quality Department, Pharmaoverseas Company, [2]Pharmaceutical Chemistry Department, College of Pharmacy, Prince Sattam Bin Abdul-Aziz University, Kingdom of Saudi Arabia, [3]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt, [4]Analytical Chemistry Department, Faculty of Pharmaceutical Sciences and Pharmaceutical industries, Future University, Egypt

Two sensitive and selective methods were developed for the simultaneous determination of four commonly used non-steroidal anti-inflammatory drugs (NSAIDs), namely; paracetamol (PCM), diclofenac sodium (DCF), ibuprofen (IBP), and indomethacin (IND) in wastewater effluents. The first method used HPLC for the determination of the studied drugs using a mobile phase consisting of phosphate buffer (pH 3.0) and acetonitrile at a flow rate of 1 mL/min. in gradient elution mode and detection at 220 nm. The separation process was performed on BDS Hypersil Cyano column (250 x 4.6 mm, 5 µm). The second method was a TLC-densitometric one which was performed using n-Hexane: ethyl acetate: acetic acid in the ratio (6:3.5:0.5) as a developing system. The proposed chromatographic methods were successfully applied for the selective determination of the four studied drugs in simulated and real pharmaceutical wastewater samples after their solid-phase extraction.

Keywords: Non-steroidal anti-inflammatory drugs. HPLC. Wastewater. TLC. SPE.

INTRODUCTION

Effluents from pharmaceuticals industry wastewater are considered one of the most serious sources of pollution in the environment. The rhythm of the modern lifestyle has made non-steroidal anti-inflammatory drugs inevitable and they are relatively abundant even in public stores and markets led their generics to the vanguard of the pharmaceutical industry. Therefore, the presence of these drugs in the industrial wastewater can prove a profound industrial leakage into the surrounding environment, that could seriously alter the ecosystem (Lindner, Umezawa 2008). Acute and chronic damages caused by the long term exposure of lower concentration of complex pharmaceutical mixtures on many organisms (Crane *et al.*, 2006; Quinn *et al.*, 2008) changes in the behavior (Gaworecki, Klaine 2008; Stanley *et al.*, 2007), accumulation in tissues (Brooks *et al.*, 2003), reproductive damage (Nentwig 2007) and cell proliferation inhibition (Pomati *et al.*, 2006). A 2014 report by UK Water Industry Research found that in most of 160 sewage treatment works studied, several common drugs were present in the final effluent in concentrations high enough to potentially affect ecosystems. The drugs included anti-inflammatories ibuprofen and diclofenac (Weber *et al.*, 2014). Diclofenac exposure in concentration ranges commonly found in the environment has been reported to cause adverse effects to brown trout, affecting kidney as well as selected immune parameters (Hoeger *et al.*, 2005). Diclofenac has proved to be highly toxic

***Correspondence:** M. A.R. Korashy. Mobile: +2 01093556454. Email: Mohamed_korashy2007@yahoo.com. **List of authors:** Sherif A. Abdel Gawad: sagawad@yahoo.com 0112366633. Nagiba Yehia Hassan: nagiba.hassan@pharma.cu.edu.eg 01111859054. M. AbdelKawy: dr.mohabdelkawy@hotmail.com 01005002155

for vultures, fish rainbow, and cattle's (Kreisberg, 2005). Additional side effects of diclofenac have been observed in humans in the liver with degenerative and inflammatory alterations, in the lower gastrointestinal tract, and the esophagus.

Ibuprofen inhibited the growth of duckweed, L. minor after 7 days of exposure at all concentrations tested. The strongest effect was observed at 1000 µg/L where a 25% reduction over the control was observed (Pomati *et al.*, 2004).

DCF, IBP, and IND have been detected in the samples collected at the Guarapiranga dam in December 2012. which is used as a source for drinking water after treatment at concentrations (28.73 - 30.25 ng/L), (166.70 - 244.73 ng/L), and (36.77 - 47.56 ng/L) respectively (Castello *et al.*, 2018).

PCM, DCF, IBP have been detected in the samples collected from 238 sites from all over France at concentrations reached 443 ng/ L, 16 ng/ L, and 19 ng/ L respectively(Bouissou-Schurtz *et al.*, 2014)

Paracetamol (PCM) is N-(4-hydroxyphenyl)acetamide, diclofenac sodium (DCF) is [O-(2, 6-dichlorophenyl)-amino-phenyl] acetate, ibuprofen (IBP) is (RS)-2-(4-(2-Methylpropyl) phenyl) propanoic acid and indomethacin (IND) is 2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl}acetic acid. They are the most familiar NSAIDs that act by the inhibition of cyclooxygenase enzyme which leads to the anti-inflammatory, analgesic, and antipyretic pharmacological effects. They are commonly formulated in different dosage forms either as single or multi-component preparations with other ingredients, Figure 1 (Sweetman, 2014; Moffat *et al.*, 2011).

Literature survey revealed different methods for the determination of the mentioned drugs either in single component preparations or in combination with other ingredients by employing different analytical techniques, that included spectrophotometric (Fadhil Ali *et al.*, 2015; Mathew *et al.*, 2013; Matin *et al.*, 2005; Pavan Kumar *et al.*, 2012) chromatographic (Alsirawan *et al.*, 2013; Devi *et al.*, 2013; Farid, Abdelaleem 2016; Nakov *et al.*, 2015; Panusa *et al.*, 2007; Szeitz *et al.*, 2010; Vemula, Sharma, 2014; Yilmaz, Ciltas, 2015; Zhao *et al.*, 2006), chemiluminescence (Mervartová *et al.*, 2007), colorimetric (Olajire *et al.*, 2006), electrochemical methods of analysis (Santini *et al.*, 2006; Swaroopa Rani, 2015); however, neither of the mentioned methods discussed the simultaneous determination of the four drugs in pharmaceutical wastewater.

Detection and monitoring of the studied drugs in pharmaceutical industrial wastewater is critical, as it affects health even if present at trace levels in the environmental wastewater.

Before applying the techniques, removal of the matrix effect and pre-concentration of the target analytes should be carried out by sample preparation and extraction. (Ibrahim *et al.*, 2017; Şahin *et al.*, 2007; Vera-Candioti *et al.*, 2008)

The current work aims to develop sensitive and selective chromatographic methods for simultaneous determination of PCM, DCF, IBP, and IND in industrial wastewater and laboratory prepared mixtures containing the mentioned drugs. The actual wastewater samples were subjected to SPE for pretreatment prior to the application of chromatographic techniques

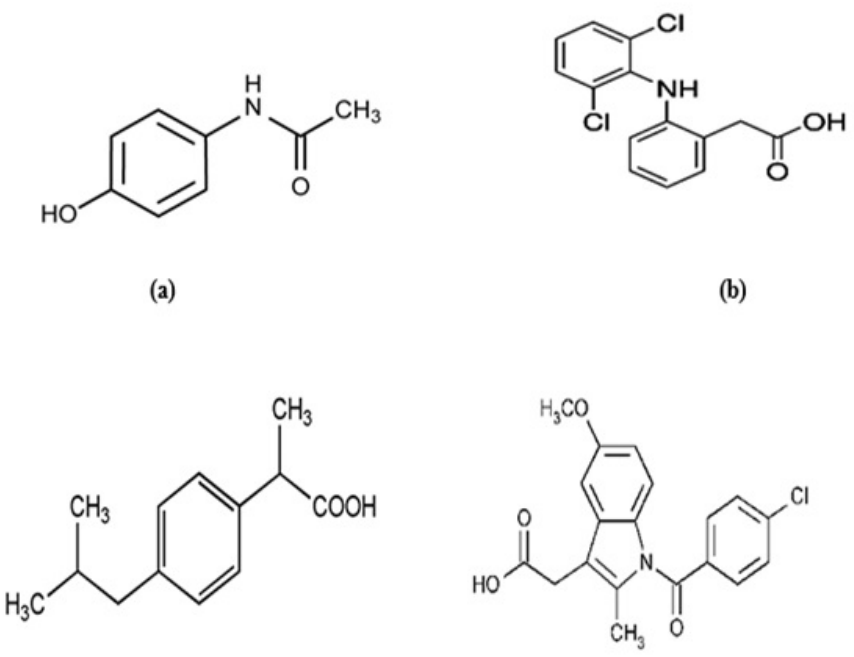

FIGURE 1 - Chemical structure of (a) PCM, (b) DCF, (c) IBP and (d) IND.

EXPERIMENTAL

Instruments

The SPE experiment was carried out using Oasis HLB (200 mg, 6ml) (Agilent Technologies, USA).

HPLC method was carried out using Agilent chromatograph 1100 series equipped with a quaternary pump (G1311A), a 20 µL loop injector, and a variable wavelength UV-visible detector (G1314A). Data acquisition was performed using Agilent Chemstation software (version 2.21).

TLC automatic sample applicator equipped with 100 µL syringe (Camag Linomat 5, Switzerland) and a TLC scanner 3 (Camag, Switzerland) was employed for preparation and measurement of TLC plates respectively. A UV lamp was used for the visualization of TLC plates.

Chemicals and reagents

Standard PCM, DCF, IBP, and IND materials were kindly supplied by an Egyptian international pharmaceutical industrial company (E.I.P.CO,10th of Ramadan City-Industrial Area -Egypt) and their percentage purity was found to be 99.92±0.37%, 99.82±0.54%, 99.92±0.37%, and 99.50±0.58%, respectively, according to official British pharmacopeia methods (The British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare Products RegulatoryAgency(MHRA) 2015).

Potassium dihydrogen orthophosphate, sulphuric acid, and orthophosphoric acid were purchased from ADWIC (Egypt). Ethyl acetate, acetic acid methanol, and acetonitrile were purchased from Sigma-Aldrich (Germany). N-Hexane was purchased from El-Nasr Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo-Egypt, and distilled water was obtained from Aquatron Automatic Water Still (A4000D, UK). All reagents used were of pure analytical grade.

Phosphate buffer pH 3 was prepared by dissolving 34 g of Potassium dihydrogen orthophosphate in 250 mL distilled water, pH was adjusted using orthophosphoric acid.

TLC aluminum plates (20×10 cm) precoated with silica gel 60 F254 were obtained from Merck, Germany.

Sampling and sample preparation

Standard solutions

Stock standard solutions of concentration 1 mg/mL of each drug were prepared in methanol.

Working standard solutions of concentration 100 µg/mL of each drug were prepared in methanol from the stock standard solution by transferring 5 mL of stock standard solutions to 50-ml measuring flasks separately and completed to the mark with methanol. The prepared mixtures were extracted by the proposed SPE before injection to HPLC and TLC.

Laboratory prepared mixtures

Nine laboratories prepared mixtures containing different amounts of PCM, DCF, IBU and IND were prepared by transferring aliquots of stock standard solutions 1 mg/mL of each drug into 25-mL measuring flasks and the volumes were completed to the mark with tap water then the prepared mixtures were analyzed using the proposed methods after SPE.

Sample collection and storage

Wastewater samples were collected from pharmaceutical industries and filtered using a Whatman filter paper Grade 42 then a 0.45 µm nylon membrane filter (Sigma Aldrich) to eliminate fine particulate matter, then the samples were placed in amber glass bottles and refrigerated at 4ºC to avoid any deterioration (Turiel *et al*., 2005) until SPE

SPE procedure

SPE was carried out using Oasis HLB cartridge tested at pH 7.0. Initially, the SPE sorbent was pre-conditioned with 6 mL methanol and 5 mL deionized water (HPLC-grade).

Application of SPE to wastewater samples

Aliquots of 100 ml of the samples (pH adjusted to 7.0 with H_2SO_4 2 N) were loaded onto the cartridge. Then the cartridge was washed using 5 ml water to remove any unbound substances and reduce interference. Finally, retained drugs were eluted from the cartridge with 10 ml MeOH at 1 mL/min, (Gómez *et al*., 2006).

To determine extraction recoveries, concentrations of the spiked wastewater matrices before and after extraction with the analytes at a concentration of 1 µL/ml were compared.

In the case of HPLC, The obtained extracts were diluted 1:4 with methanol and the obtained solutions were analyzed, but in the case of TLC, the obtained extracts were analyzed directly without dilution.

HPLC method

Analysis conditions

Following SPE, 20 µL of the samples were subjected to HPLC analysis, Chromatographic separation was performed using BDS Hypersil Cyano column (250 x 4.6 mm, 5 µm) maintained at 60ºC. The flow rate was kept at 1 mL/min and gradient elution of the mobile phase consisting of phosphate buffer pH 3.0 acetonitrile was carried out. The ratio was kept at 96:4, v/v till 7 min, then the ratio was gradually changed to 68:32, v/v till 10 minutes; then, held until 20 minutes, UV detection was performed at 220.0 nm.

Method validation

Aliquots of working standard solutions of each drug were separately transferred to 10 mL measuring flasks and the volume was completed to the mark using methanol to cover the concentration range 0.25-10.00 µg/mL. Calibration curves were constructed and regression equations were computed (Kidd, 1996).

TLC method

Analysis conditions

Suitable aliquots eluted from SPE were applied to the TLC plates. Chromatographic separation was performed on TLC aluminum plates (20 ×10 cm) precoated with silica gel 60 F254. Camag Linomat 5 applicator was used for the application of samples of drugs. Bands were applied at 5 mm intervals and 15 mm from the bottom and sides. The chromatographic chamber was saturated with the mobile phase for one hour and the plate was developed by ascending technique using n-Hexane: ethyl acetate: acetic acid (6:3.5:0.5) as a mobile phase to a distance of about 8 cm. The plate was dried in air at room temperature, detected under a UV lamp, and scanned at 254.0 nm.

Method validation

Different aliquots from working standard solution of PCM, DCF, and IND (100 µg/mL) and from stock standard solutions of IBP (1 mg/mL), were transferred into a series of 10 mL volumetric flasks, and completed to the mark with methanol to cover the range of 0.10-0.90 µg/band for PCM, DCF and IND and 1.00-9.00 µg/band for IBP. Calibration curves were constructed and regression equations were computed

RESULTS AND DISCUSSION

The current work aimed to develop sensitive and selective methods for the simultaneous determination of the residues of four commonly used NSAIDs in industrial wastewater and laboratory prepared mixtures containing the mentioned drugs. The four mentioned drugs exhibit chemical similarity which can be noticed in their overlapped spectral data, so it was inapplicable using the usual spectrophotometric method, Figure 2.

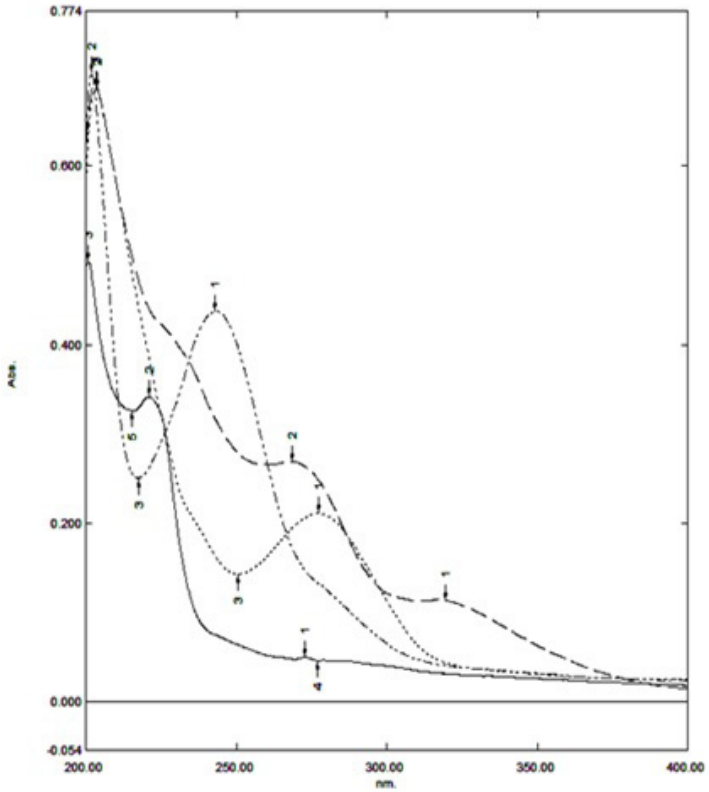

FIGURE 2 - Absorption spectra for PCM (. .), DCF (….), IBU ( ) and IND (----) measured in methanol.

For the HPLC method

For simultaneous elution of PCM, DCF, IBP, and IND peaks, acetonitrile and phosphate buffer where tried in different ratios using gradient elution to reach the optimum chromatographic conditions, the optimum composition of the mobile phase was set as phosphate buffer pH 3.0: acetonitrile with a ratio kept at 96:4, v/v till 7 min, then the ratio was gradually changed to 68: 32, v/v till 10 minutes; then, held till 20 minutes, with a flow rate of 1 mL/min and the detection was carried out at 220.0 nm The retention times were found to be 3.3, 14.4, 14.9 and 15.5 minutes for PCM, IND, DCF, and IBP, respectively, Figure 3.

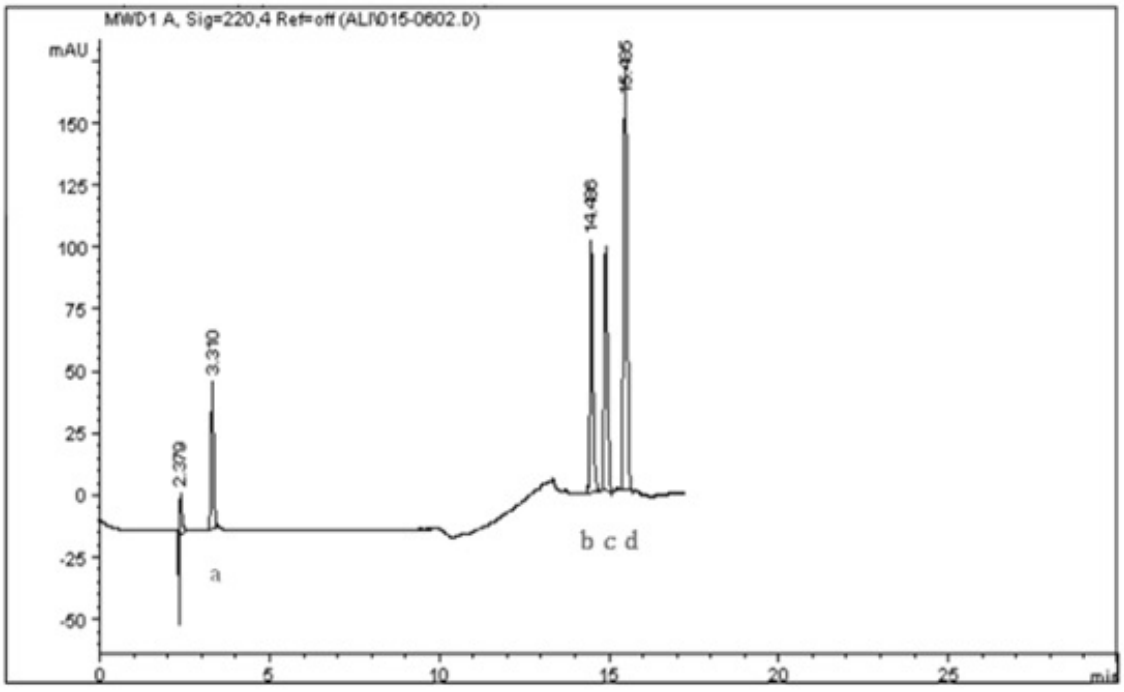

FIGURE 3 - HPLC chromatogram of (a) PCM, (b) IND, (c) DCF and (d) IBP, using the specified chromatographic conditions.

System suitability parameters were calculated, as the resolution, selectivity factors, tailing, column efficiency (as no. of theoretical plates), and capacity factors. The results obtained were shown in Table I.

Representative chromatograms of pharmaceutical wastewater samples diluted 1:4 after SPE and those spiked with 1 µg/ml, are shown in Figure 4, 5.

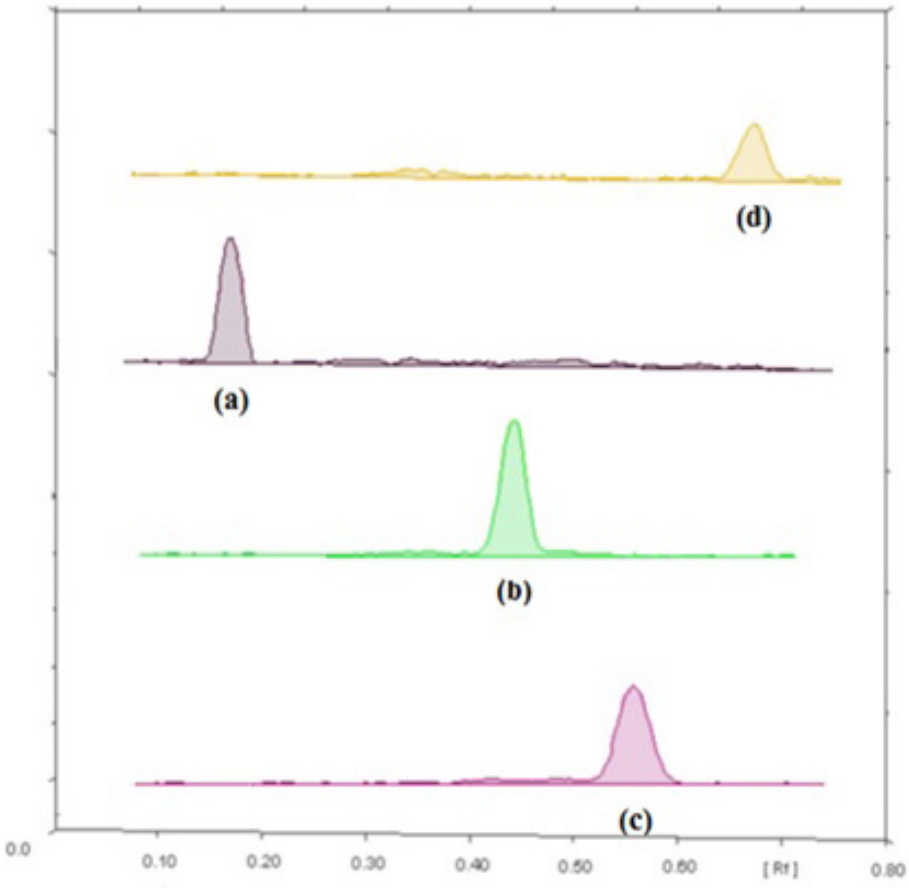

FIGURE 4 - HPLC chromatogram of the sample (1) without (b) with spiking with the analytes using the specified chromatographic conditions.

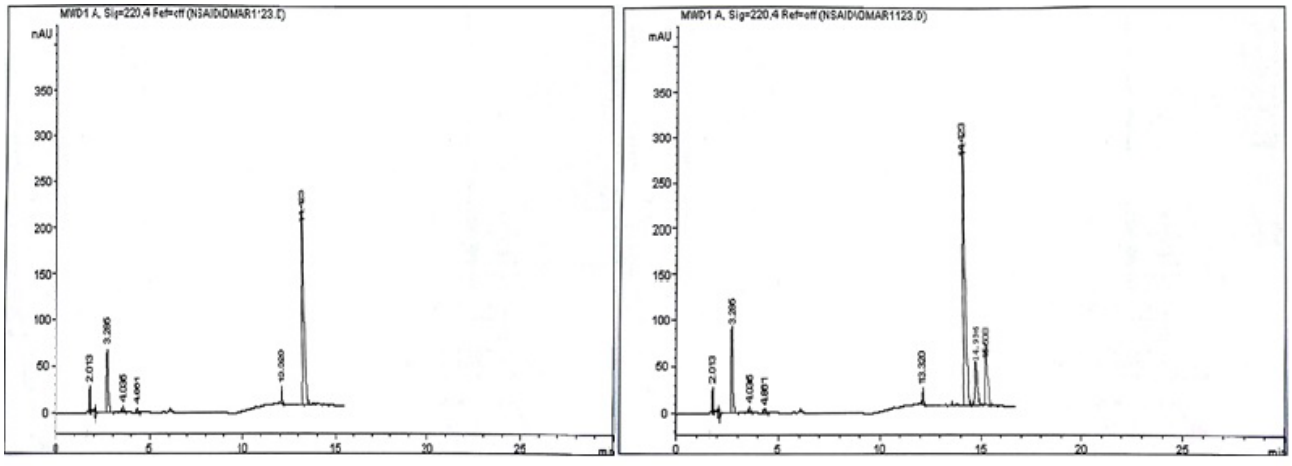

FIGURE 5 - HPLC chromatogram of the sample (2) without (b) with spiking with the analytes using the specified chromatographic conditions.

For TLC method

TLC densitometric technique is suggested for the simultaneous determination of PCM, IBP, DCF, and IND in pure samples and wastewater from pharmaceutical industries. The method is based on the difference in Rf values of each drug from the other studied drugs. The satisfactory separation was obtained by using n-Hexane: ethyl acetate: acetic acid (6:3.5:0.5, by volumes) as a developing system. The Rf values were 0.18, 0.56, 0.69 and 0.44 for PCM, DCF, IBP and IND respectively. The TLC separation is presented in Figure 6.

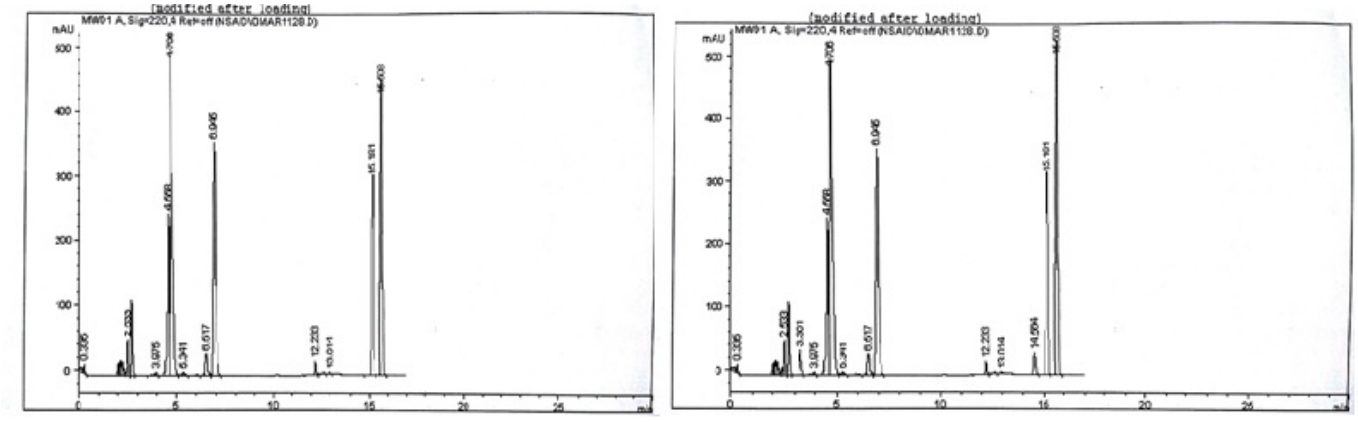

FIGURE 6 - Typical TLC chromatogram of (a) PCM, (b) IND, (c) DCF and (d) IBP.

System suitability parameters were calculated, as the resolution, selectivity factors, tailing, column efficiency (as no. of theoretical plates), and capacity factors (Rockville, 2015; Spangenberg *et al*., 2010). The results obtained were shown in Table I.

The validity of the proposed methods

The mixtures of PCM, DCF, IBP, and IND with different composition ratios were analyzed to perform recovery studies which indicate the validity and applicability of the proposed HPLC and TLC methods. The characteristic parameters and necessary statistical data of the regression equation, the limit of detection (LOD), specificity, precision, and accuracy data for HPLC and TLC techniques are collected in Table I.

TABLE I - Parameters required for system suitability test of the proposed HPLC and TLC methods

	HPLC				TLC				Reference Value
	PCM	DCF	IBP	IND	PCM	DCF	IBP	IND	
Resolution (R)*	-	4.58	5.83	136.76	-	1.26	1.30	3.00	R > 1.25
Tailing Factor (T)	0.99	0.95	0.98	0.96	-	-	-	-	T=1 for a typical symmetric peak
Capacity factor (k)**	0.68	6.52	6.81	6.31	0.18	0.56	0.69	0.44	1-10 acceptable for HPLC 0-1 acceptable for TLC
Selectivity (α) ***	-	1.03	1.04	9.34	-	3.11	3.83	2.44	> 1
Column efficiency (N)******	36097.19	416734.69	356003.92	384909.88	-	-	-	-	Increases with efficiency of the separation
Height Equivalent to theoretical Plates (HETP)	6.93×10^{-4}	6.00×10^{-5}	7.02×10^{-5}	6.50×10^{-5}	-	-	-	-	The smaller the value, the higher the column efficiency
Symmetry Factor					1.00	0.95	0.98	1.00	

* Resolution $R= 2\frac{(t_{R2}-t_{R1})}{(w_1+w_2)}$ where $t_{R2,}$ t_{R1} retention time of two adjacent peaks and $w_{1,}$ w_2 width of these peaks (for HPLC) taking PCM as a reference. $R = 2(Z_{S2} - Z_{S1})/ (W1 + W2)$ Where Z_{S2} and Z_{S1} are the quotients from the difference between the two maximum signals; and W2 and W1 are the arithmetic mean of their peak widths at the base. (for TLC) taking PCM as a reference.

** Capacity Factor $k= \frac{(t_R-t_0)}{t_0}$ where t_R retention time of the substance and t_0 is the retention time of the highly retained substance for HPLC $k = \frac{\text{distance travelled by the substance in the stationaty phase}}{\text{distanve travelled by the solvent in the mobile phase}}$ for TLC

*** Selectivity (α)= K_2/k_1 where k retention factor (Capacity Factor)

**** Column efficiency (number of theoretical plates) $N= 16\ [\frac{t_R}{w}]^2$ where t_R retention time of the substance and w width of its peak

A comparison was done between the results obtained by the proposed methods (HPLC and TLC) for the determination of the selected drugs in pure form and those obtained by the official BP methods. The calculated t and F values were less than the critical t and F values which indicated that no significant difference between the applied methods and official methods. The results obtained were shown in Table II.

TABLE II - Results of assay validation of the proposed, HPLC, and TLC methods.

Parameter	HPLC				TLC			
	PCM	DCF	IBP	IND	PCM	DCF	IBP	IND
Slope*	103.4	218.4	425.7	219.9	5963	8420	1971	9683
Intercept	9.856	24.02	45.01	27.43	819.6	673.8	1401	-837.5
Range**	0.25–15	0.2–15	0.25–15	0.25–15	0.1-0.9	0.1-0.9	1.0-9.0	0.1-0.9
Correlation coefficient (r)	0.999	0.999	0.999	0.999	0.999	0.998	0.999	0.999

(continues on the next page...)

Parameter		HPLC				TLC			
		PCM	DCF	IBP	IND	PCM	DCF	IBP	IND
Specificity (mean±SD)		99.54± 0.84	99.34± 0.64	99.26± 1.36	99.96± 1.37	99.56± 0.55	100.33± 1.93	99.53± 1.36	99.05± 0.45
Accuracy (mean±SD)		100.02± 0.86	99.77± 0.99	99.77± 1.09	99.58± 0.61	99.67± 1.72	99.76± 1.78	99.73± 0.83	99.2± 1.62
Precision (% RSD)***	**Intraday ******	0.19	0.41	0.13	0.14	0.30	0.13	0.86	0.20
	Interday*****	1.01	0.75	0.42	0.13	1.01	0.51	0.54	0.95
LOD* (µg/mL)		6.38×10^{-3}	1.51×10^{-3}	1.18×10^{-3}	2.29×10^{-3}	3×10^{-4}	7.58×10^{-4}	2.2×10^{-3}	4×10^{-4}
LOQ* (µg/mL)		1.93×10^{-2}	4.57×10^{-3}	3.58×10^{-3}	6.94×10^{-3}	9.2×10^{-4}	2.29×10^{-3}	6.9×10^{-3}	1.22×10^{-3}

* Average results of three determinations

** Concentration calculated as µg/mL for HPLC method and µg/band for the TLC method.

*** Percent relative standard deviation

**** The intraday (n = 3), RSD of three concentrations (0.5, 1, 5 µg/mL) for all the four drugs repeated three times within the day.

***** The interday (n = 3), RSD of three concentrations (0.5, 1, 5 µg/mL) for all the four drugs repeated three times in three successive days.

On the other hand, another statistical comparison of the results using the proposed methods was done using One way Repeated ANOVA analysis to compare the concentration of the studied drugs in the nine laboratory prepared mixtures, as shown in Table III. There was no significant difference at $\alpha = 0.05$ Table IV.

TABLE III - Statistical treatment of the results obtained by the suggested HPLC and TLC methods in comparison with the different methods in pure form.

Item	Official method*				HPLC				TLC			
	PCM	DCF	IBP	IND	PCM	DCF	IBP	IND	PCM	DCF	IBP	IND
Mean	99.92	99.92	99.82	99.5	99.54	99.34	99.26	99.96	99.56	99.53	100.33	99.05
SD	0.37	0.37	0.54	0.58	0.84	0.64	1.36	1.37	0.55	1.36	1.93	0.45
Variance	0.14	0.14	0.29	0.34	0.7	0.41	1.86	1.87	0.3	1.84	3.72	0.2
N	5	5	5	5	5	5	5	5	5	5	5	5
Student's t-test (2.306)**	-	-	-	-	0.93	1.75	0.85	0.69	1.22	0.62	0.57	1.37
F value (6.3882)**	-	-	-	-	5.11	2.99	6.38	5.56	2.19	13.44	12.76	1.68

*Official BP methods were titration method for PCM, IBP, IND and potentiometric titration method for DCF

**Student's t-test and F values represent the corresponding tabulated values of t and F at P=0.05.

TABLE IV - One way Repeated ANOVA statistical analysis of the results obtained by applying the proposed method for the determination of PCM, DCF, IBP, and IND within 95% confidence limit.

Results of One Way Repeated Anova							Comparison Between Techniques*			
Source of variation		Sum of Squares	df	Mean Square	F	Sig.	(I) Techniques	(J) Techniques	Mean Difference (I-J)	Sig.[a]
PCM	Between Techniques	16.06	1.00	16.06	2.28	0.17	1.00	2.00	-1.89	0.17
	Error(Between Samples	418.00	8.00	52.25						
DCF	Between Techniques	1.39	1.00	1.39	0.43	0.53	1.00	2.00	0.56	0.53
	Error(Between Samples	179.44	8.00	22.43						
IBP**	Between Techniques	-	-	-	-	-	-	-	-	-
	Error(Between Samples	-	-	-						
IND	Between Techniques	18.00	1.00	18.00	3.27	0.11	1.00	2.00	-2.00	0.11
	Error(Between Samples	1037.78	8.00	129.72						

*1=HPLC, 2=TLC

APPLICATION

The chromatographic methods were successfully applied for the determination of real wastewater samples from pharmaceutical industries containing the studied drugs after pretreated by SPE-technique. The proposed methods were suitable for the determination of the concentration of the studied drugs except for the TLC technique in case of determination of IBP which was only suitable for high concentrations of IBP due to the high limit of determination, as shown in Table V.

TABLE V - Determination of PCM, DCF, IBP, and IND in wastewater samples from pharmaceutical industries by the proposed HPLC and TLC methods.

	HPLC				TLC			
	PCM	DCF	IBP	IND	PCM	DCF	IBP	IND
Sample1	3.9	-	-	1.5	3.64	-	-	1.73
Sample2	-	3.4	2.9	-	-	3.1	-	-

(continues on the next page...)

	HPLC				TLC			
	PCM	**DCF**	**IBP**	**IND**	**PCM**	**DCF**	**IBP**	**IND**
Sample3	2.8	3.1	-	-	2.75	3.3	-	-
Sample4	3.5	-	1.25	-	3.8	-	-	-
Sample5	-	-	-	1.4	-	-	-	1.65

* Concentration calculated as μg/mL for HPLC and TLC method.**V**

CONCLUSION

The novelty of this work lies in HPLC and TLC methods for the direct, simple, and sensitive simultaneous quantification of PCM, DCF, IBP, and IND in different samples, especially in industrial wastewater and in the case of complex mixtures, with sample pretreatment for cleanup and/or pre-concentration. This constitutes a major advantage for the proposed techniques as it ensures the sensitivity and suitability of the proposed techniques for environmental analysis. Also, cross-validation between the proposed methods was carried out, where the perfect agreement between the results of chromatographic assays proved by the results obtained from One way Repeated ANOVA analysis which indicated that no significant difference between the applied methods.

Also, validation of the proposed methods was carried out, where good agreement between the results of these methods and official methods proved by obtaining results indicated that no significant difference between the applied methods.

HPLC method has the advantage of high sensitivity, wide working concentration ranges, and lower limits of detection but the TLC technique is simpler and less expensive.TLC technique was not recommended for the determination of IBP due to its high limit of determination. The proposed methods are rapid, selective, and characterized by their good recoveries and have crucial importance in monitoring the environmental pollution with the studied non-steroidal anti-inflammatory drug residues, where the presence of these substances in the environment could alter the ecosystem seriously.

Efforts have been done for the evaluation and reduction of pharmaceutical contamination of the environment. Nevertheless, only preliminary measures aimed at protecting the environment from the adverse effects of pharmaceutical pollutants are in place. These measures must be supplemented by integrated programs. Projects should facilitate identification, prioritization, and evaluation of human health and environmental risk; close the gap in knowledge on the environmental behavior of pharmaceuticals; improve the scientific basis of regulatory decision; harmonization of the water protection legislation. There is also a need for more screening actions regarding the prevention of an uncontrolled discharge of pharmaceuticals in the environment.

REFERENCES

Alsirawan MB, Mohammad MA, Alkasmi B, Alhareth K, El-Hammadi M. Development and validation of a simple HPLC method for the determination of ibuprofen sticking onto punch faces. Int J Pharm Pharm Sci. 2013;5(4):227-31.

Bouissou-Schurtz C, Houeto P, Guerbet M, Bachelot M, Casellas C, Mauclaire A-C, et al. Ecological risk assessment of the presence of pharmaceutical residues in a French national water survey. Regulatory Toxicology and Pharmacology [Internet]. Academic Press; 2014. Aug 1 [cited 2019 Mar 11];69(3):296–303. Available from: https://www.sciencedirect.com/science/article/pii/S0273230014000695

Brooks BW, Turner PK, Stanley JK, Weston JJ, Glidewell EA, Foran CM, et al. Waterborne and sediment toxicity of fluoxetine to select organisms. Chemosphere. 2003;52(1):135-42.

Castello M, Pais N, De Souza Nascimento E. Guideline values and human risk assessment for the presence of anti-inflammatory drugs remaining in drinking water after lab scale treatment. J. Pharm. Sci [Internet]. 2018 [cited 2019 Mar 8];54(1):17320. Available from: http://dx.doi.org/10.1590/s2175-97902018000117320

Crane M, Watts C, Boucard T. Chronic aquatic environmental risks from exposure to human pharmaceuticals. Sci Total Environ. 2006;367(1):23-41.

Devi TAP, Setti A, Srikanth S, Nallapeta S, Pawar SC, Rao JV. Original article Method development and validation of paracetamol drug by RP-HPLC. J Med Allied Sci. 2013;3(1):8-14.

Fadhil Ali K, Rasool Mahmood Albakaa A, Hussein Ali Z. New assay method UV spectroscopy for determination of Indomethacin in pharmaceutical formulation. J Chem Pharm Res. 2015;7(4):1591-1596.

Farid NF, Abdelaleem EA. HPTLC Method for the Determination of Paracetamol, Pseudoephedrine, and Loratidine in Tablets and Human Plasma. Journal of chromatographic science [Internet]. Oxford University Press; 2016 Apr [cited 2016 Oct 18];54(4):647-652. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26762956

Gaworecki KM, Klaine SJ. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. Aquat Toxicol. 2008;88(4):207-213.

Gómez MJ, Petrović M, Fernández-Alba AR, Barceló D. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. J Chromatogr A. 2006;1114(2):224-233.

Hoeger B, Köllner B, Dietrich DR, Hitzfeld B. Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (Salmo trutta f. fario). Aquat Toxicol. 2005;75(1):53-64.

Ibrahim HK, Abdel-Moety MM, Abdel-Gawad SA, Al-Ghobashy MA, Kawy MA. Validated electrochemical and chromatographic quantifications of some antibiotic residues in pharmaceutical industrial wastewater. Environmental Science and Pollution Research [Internet]. Springer Berlin Heidelberg; 2017 Mar 14 [cited 2018 Jan 24];24(8):7023-7034. Available from: http://link.springer.com/10.1007/s11356-016-8340-3

Kidd D. International Conference on Harmonization. International Conference on Harmonization [Internet]. 1996 [cited 2016 Nov 1];4(1):183-206. Available from: http://www.ich.org/home.html

Kreisberg J. Ecological healing and the web of life. EXPLORE: The Journal of Science and Healing [Internet]. Elsevier; 2005 Mar [cited 2017 Feb 24];1(2):133-135. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1550830704000242

Lindner E″, Umezawa Y. Performance evaluation criteria for preparation and measurement of macro- and micro fabricated ion selective electrodes. Pure Appl. Chem. 2008;80(1):85-104.

Mathew M, Ashokan AS, Puthusseri S. Development and validation of UV spectrophotometric methods for simultaneous estimation of tolperisone hydrochloride and diclofenac sodium in tablet dosage form. International Journal of Pharmacy and Biological Sciences [Internet]. 2013 [cited 2016 Oct 18];3(4):42-48. Available from: www.ijpbs.com

Matin AA, Farajzadeh MA, Jouyban A. A simple spectrophotometric method for determination of sodium diclofenac in pharmaceutical formulations. Il Farmaco. 2005;60(10):855-858.

Mervartová K, Polášek M, Calatayud JM. Sequential injection analysis (SIA)-chemiluminescence determination of indomethacin using tris[(2,2′-bipyridyl)]ruthenium(III) as reagent and its application to semisolid pharmaceutical dosage forms. Analytica Chimica Acta. 2007;600(1):114-121.

Moffat AC, Osselton MD, Widdop B, Watts J. Clarke's analysis of drugs and poisons. Fourth Edi.. 2011.

Nakov N, Petkovska R, Ugrinova L, Kavrakovski Z, Dimitrovska A, Svinarov D. Critical development by design of a rugged HPLC-MS/MS method for direct determination of ibuprofen enantiomers in human plasma. Journal of Chromatography B. 2015;992:67-75.

Nentwig G. Effects of Pharmaceuticals on Aquatic Invertebrates. Part II: The Antidepressant Drug Fluoxetine. Archives of Environmental Contamination and Toxicology [Internet]. Springer-Verlag; 2007 Feb 7 [cited 2017 Feb 24];52(2):163-170. Available from: http://link.springer.com/10.1007/s00244-005-7190-7

Olajire AA, Olakunle SI, Ajibola AO. Novel colorimetric assay of indomethacin using 4-carboxyl-2,6-dinitrobenzene diazonium ion. Acta Pharm. 2006;56:189-202.

Panusa A, Multari G, Incarnato G, Gagliardi L. High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products. Journal of Pharmaceutical and Biomedical Analysis. 2007;43(4):1221-1227.

Pavan Kumar SR, Bhuvan Kumar G, Sekhar TC, Murthy SB. Spectrophotometric Determination of Paracetamol Using Sodium bismuthate as Chromogen. Int. J. Res. Chem. Environ. International Journal of Research in Chemistry and Environment. 2012;2(2):231-235.

Pomati F, Castiglioni S, Zuccato E, Fanelli R, Vigetti D, Rossetti C, et al. Effects of a complex mixture of therapeutic drugs at environmental levels on human embryonic cells. Environmental science & technology [Internet]. 2006 Apr 1 [cited 2017 Feb 24];40(7):2442–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16646487

Pomati F, Netting AG, Calamari D, Neilan BA. Effects of erythromycin, tetracycline, and ibuprofen on the growth

of Synechocystis sp. and Lemna minor. Aquatic Toxicol. 2004;67(4):387396.

Quinn B, Gagné F, Blaise C. An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata. Sci Total Environment. 2008;389(2): 306-314.

Rockville. The Pharmacopoeia of the United States - National Formulary USP 39-NF 34 [Internet]. 2015 [cited 2017 Mar 31]. Available from: http://www.pharmacopeia.cn/v29240/usp29nf24s0_c621_viewall.html

Şahin S, Demir C, Güçer Ş. Simultaneous UV-vis spectrophotometric determination of disperse dyes in textile wastewater by partial least squares and principal component regression. Dyes Pigm. 2007;73(3):368-376.

Santini AO, de Oliveira JE, Pezza HR, Pezza L. A new potentiometric ibuprofenate ion sensor immobilized in a graphite matrix for determination of ibuprofen in tablets. Microchem J. 2006;84(1):44-49.

Spangenberg B, Poole CF, Weins C. Theoretical Basis of Thin Layer Chromatography (TLC). Quantitative Thin-Layer Chromatography [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2010 [cited 2017 Mar 31]. p. 13-52. Available from: http://link.springer.com/10.1007/978-3-642-10729-0_2

Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW. Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. Chemosphere. 2007;69(1):9-16.

Swaroopa Rani GN. Polarographic determination of Paracetamol by Calibration method using Fuchsin and different supporting electrolytes. Int Res J Sci Eng. 2015;3(34):156-165.

Sweetman SC. The Complete Drug Reference [Internet]. 36th editi. London,United Kingdom: Pharmaceutical Press; 2014 [cited 2018 Jan 25]. Available from: https://www.abe.pl/html/files/2014047-DMO-Martindale_2014.pdf

Szeitz A, Edginton AN, Peng HT, Cheung B, Riggs KW. A Validated Enantioselective Assay for the Determination of Ibuprofen in Human Plasma Using Ultra Performance Liquid Chromatography with Tandem Mass Spectrometry (UPLC-MS/MS). Am J Anal Chem [Internet]. Scientific Research Publishing; 2010 [cited 2016 Oct 18];01(02):47–58. Available from: http://www.scirp.org/journal/PaperDownload.aspx?DOI=10.4236/ajac.2010.12007

The British Pharmacopoeia Commission Secretariat of the MedicinesandHealthcareProductsRegulatoryAgency(MHRA). British Pharmacopeia. I,II. London,United Kingdom: (The Stationery Office); 2015.

Turiel E, Bordin G, Rodríguez AR. Determination of quinolones and fluoroquinolones in hospital sewage water by off-line and on-line solid-phase extraction procedures coupled to HPLC-UV. J Sep Sci [Internet]. WILEY-VCH Verlag; 2005 [cited 2016 Oct 18];28(3):257-267. Available from: http://doi.wiley.com/10.1002/jssc.200400018

Vemula V, Sharma P. Gradient High Performance Liquid Chromatography Method Development and Validation for Simultaneous Determination of Phenylephrine and Ibuprofen in Tablet Dosage Form. Trop J Pharm Res [Internet]. Faculty of Pharmacy, University of Benin; 2014 [cited 2016 Oct 18];13(6):967-974. Available from: http://www.ajol.info/index.php/tjpr/article/view/107722

Vera-Candioti L, Gil García MD, Martínez Galera M, Goicoechea HC. Chemometric assisted solid-phase microextraction for the determination of anti-inflammatory and antiepileptic drugs in river water by liquid chromatography-diode array detection. J Chromatogr A. 2008;1211(1–2):22-32.

Weber F-A, Aus Der Beek T, Bergmann A, Carius A, Grüttner G, Berlin G, et al. Pharmaceuticals in the environment-the global perspective Occurrence, effects, and potential cooperative action under the Strategic Approach to International Chemicals Management (SAICM) [Internet]. 2014. Available from: www.stoffers-steinicke.de

Yilmaz B, Ciltas U. Determination of diclofenac in pharmaceutical preparations by voltammetry and gas chromatography methods. J Pharm Anal. 2015;5(3):153-160.

Zhao S, Bai W, Yuan H, Xiao D. Detection of paracetamol by capillary electrophoresis with chemiluminescence detection. Anal Chim Acta. 2006;559(2):195-199.

Received for publication on 26th August 2018
Accepted for publication on 01st April 2019