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Article

Investigation of the Environmental Transport of Human Pharmaceuticals to Surface Water: A Case Study of Persistence of Pharmaceuticals in Effluent of Sewage Treatment Plants and Hospitals in Malaysia

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The present work reports the occurrence and monitoring of 11 pharmaceuticals (i.e., caffeine, prazosin, enalapril, carbamazepine, nifedipine, gliclazide, levonorgestrel, simvastatin, hydrochlorothiazide, diclofenac-Na and mefenamic acid) in surface water and in the influent and effluent of sewage treatment plants (STPs) and hospitals (HSPs). A total of 105 water samples were analyzed using solid-phase extraction combined with liquid chromatography-time-of-flight/mass spectrometry (SPE-LC-TOF/MS). The mean concentrations of the detected pharmaceuticals in STP influent and effluent ranged from < limit of quantification (LOQ) to 3909 ng L^{-1} and 12 to 577 ng L^{-1}, respectively. The mean concentrations of the detected pharmaceuticals in the hospital influent and effluent ranged from 28 to 1644 ng L^{-1} and 20 to 1540 ng L^{-1}, respectively. The highest concentration detected in the sampling points was 9099 ng L^{-1} for caffeine in influent STP. The presence of prazosin has never been reported before in literature. In this study, prazosin was detected in all studied samples, and the highest concentration was 525 ng L^{-1} in influent STP. Chemometric analysis was used to assess the presence of pharmaceuticals in samples.

Keywords: pharmaceuticals in water, chemometric analysis, monitoring of pharmaceuticals, pharmaceutical removal

Introduction

Presence of a large number of pharmaceuticals in different bodies of water may affect the purity of drinking water. Given that surface water is the most affected, pharmaceuticals may initially pose a problem to water utilities that use surface water as a water resource for drinking-water production. Trace amounts of emerging organic contaminants (EOCs), such as pharmaceuticals and hormones, can enter the environment. In the 1990s, researchers began to identify and quantify these EOCs in influent and effluent of sewage treatment plants (STPs), surface water, and drinking water in Asia, Europe, the United States, and Canada.[1-11]

Presence of pharmaceuticals in STPs was discovered in the 1970's, in which two compounds namely, clofobric acid and salicylic acid, were detected in a municipal sewage.[12] Since then, research in the field has expanded dramatically, and almost 100 pharmaceuticals or their metabolites have been detected in water samples.[13]

Various studies have recently shown that a large number of pharmaceuticals are ubiquitously present in surface water that are influenced by influent and effluent of STPs and influent and effluent of hospitals.[13-16] Although some studies have detected pharmaceuticals or their metabolites in drinking water, these compounds were of minor concern

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to humans because of their extremely low concentrations (in the range of few ng L^{-1}) compared with therapeutic doses (in the ranges of milligrams).[15] Presence of these compounds in drinking water is still a sign of contamination originating from sewage, so the analysis of low concentrations (i.e., ng L^{-1}) of pharmaceuticals found in water samples requires highly sensitive and selective analytical methods in detecting pharmaceuticals in samples. Solid phase extraction (SPE) cartridges are the most appropriate solution to meet this requirement followed by liquid chromatography combined with mass spectrometry.[2,17-19] The structure, therapeutic classes and physicochemical properties for all studied pharmaceuticals are presented in Figure 1 and Table 1. In Malaysia, research in this field is still at its infancy, so conducting such study is required to collect more information about Malaysian aquatic environment.

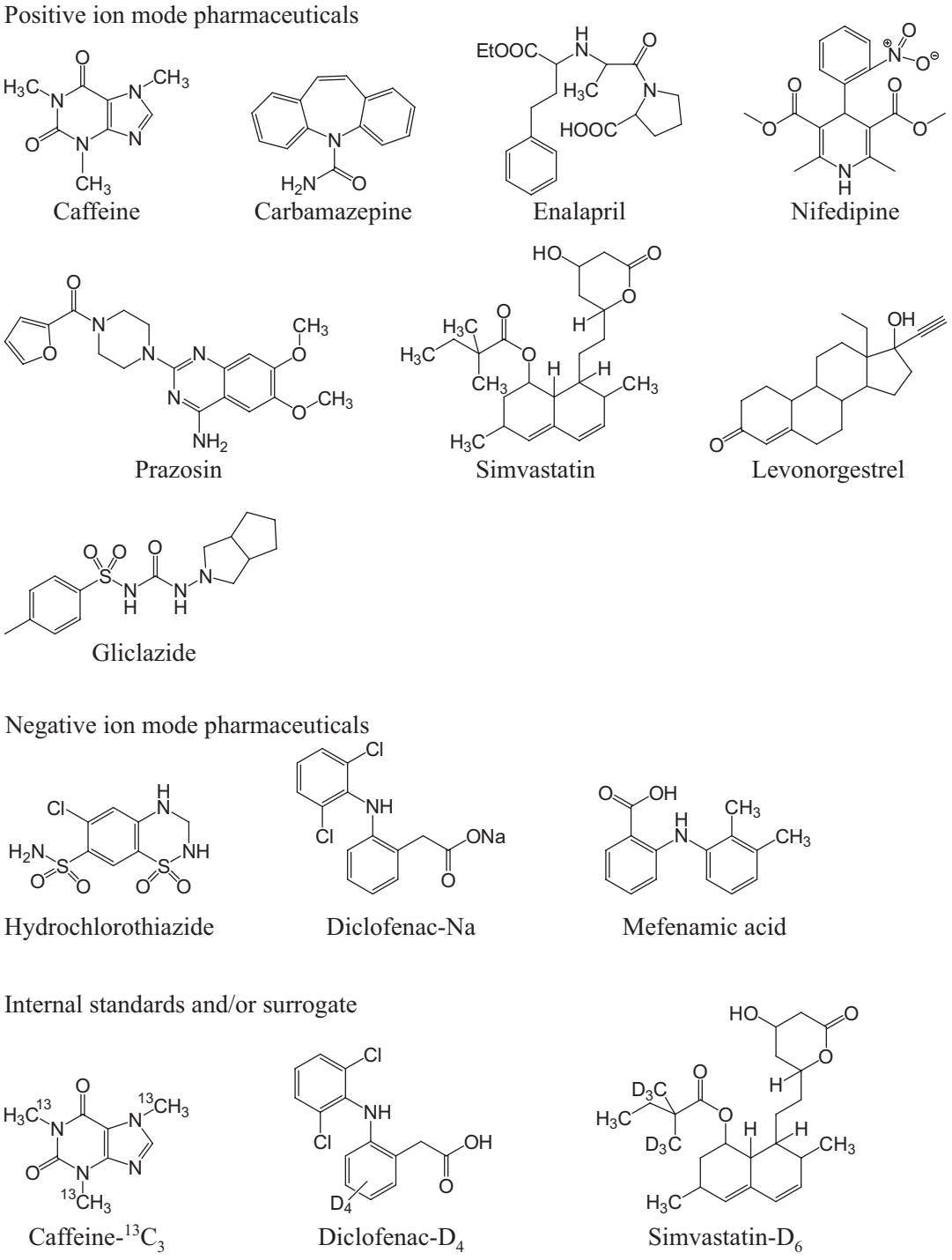

Figure 1. Chemical structure of the studied pharmaceuticals and internal and/or surrogate standards.

Table 1. Physicochemical properties of the selected pharmaceuticals[20-26]

Therapeutic use (ATC code)	Compound	CAS number	Trade names	M_{wt} / (g mol^{-1})	pKa	logK_{ow}
Calcium channel blockers (C08)	Nifedipine (C08CA05)	21829-25-4	Adalat, Procardia	346.335	14	–0.07
Diabetics (A10)	Gliclazide (A01BB09)	21187-98-4	NA	323.412	6.5	1.28
Renin-angiotensin (C09)	Enalapril.malate (C09AA02)	75847-73-3	Vasotec	376.446	3.0	0.49
Lipid modifying agents (C10)	Simvastatin (C10AA01)	79902-63-9	Zocor	418.56	13.9	2.45
Anti-inflammatory (M01)	Diclofenac-Na (M01AB05)	15307-79-6	Solaraze, Voltaren	318.149	< 1	2.20
Antiepileptics (N03)	Carbamazepine (N03AF01)	298-46-4	Tegretol	236.2686	NA	3.48
Diuretics (C03)	Hydrochlorothiazide-HCL (C03AA03)	58-93-5	Microzide	297.739	13.49	4.68
Antihypertensive (C02)	Prazosin (C02CA01)	19216-56-9	Minipress	383.4011	7.9	–0.07
Sex hormones (G03)	Levonorgestrel (G03AA07)	797-63-7	Plan B	312.4458	NA	2.12
Anti-inflammatory (M01A)	Mefenamic acid (M01AG01)	61-68-7	Ponstan	241.2851	4.2	1.13
Nervous system (N06B)	Caffeine (N06BC01)	58-08-2	NA	194.1906	4.2	5.12

NA: not available.

Experimental

Material and methods

Study site

All samplings were performed in Negeri Sembilan, one of Malaysian's 13 states, which lies on the western coast of Peninsular Malaysia, immediately south of Kuala Lumpur and borders Selangor on the north, with Pahang in the east and Malacca and Johor to the south. The population of Negeri Sembilan is approximately 1.0 million, and the total area is approximately 6686 km^2. Samples were collected from four STPs, three hospital (HSPs), and two receiving surface water (SW) in the state. A total of 13 sampling points, in the present study, include the SW as well as the influents and effluents of STPs and HSPs as presented in Figure S1 in the Supplementary Information (SI) section.

Sample collection

Table 2 shows some characteristics of the four STPs and three HSPs. Samples were also collected from the surface water SW at two points (SW STP 1 and SW HSP 2). The first three STPs (STP1, STP2, and STP3) are the main sources of sewage effluent in the Langat River, and STP4 is considered the biggest source in the Negeri Sembilan. Sampling was conducted from May to December 2013 (eight months). All samples were collected in 1 L amber glass bottles with Teflon-lined caps to ensure sample integrity using a high-density polyethylene plastic bucket previously rinsed with distilled water and MeOH. The bottle head space was kept to a minimum by completely filling the bottles. The bottles were rinsed in the field twice with the sample and completely filled on the third sampling. Disposable gloves were used by the sampler to prevent any personal care products from contaminating the sample bottles. The collection of samples from influent and effluent of sewage treatment plants is to evaluate the treatment performance and is to explain which compounds are still persistent after treatment.

Method of analysis

The pharmaceuticals present in the collected samples were analyzed using a previously developed and validated method based on SPE, followed by liquid chromatography-time-of-flight/mass spectrometry (LC-TOF/MS).[27] Briefly; the samples were preserved by adding 1 g L^{-1} of sodium azide to prevent microbial degradation. Aliquots of 100, 250, 500, and 1000 mL were taken from the STP and HSP influent, STP and HSP effluent, SW, and deionized water (DIW), respectively. The aliquots were then passed through a 0.7 µm Whatman GF/F filter (UK) to remove particulate matter present in the water samples. Subsequently, the samples were stored at 4 °C to minimize the degradation of pollutants until the samples were extracted using SPE. The pharmaceuticals were extracted from the aqueous samples using Oasis HLB (3 cc, 60 mg) SPE cartridges by means of a 10-sample GAST

Table 2. Information about the studied STPs and HSPs

Sewage treatment plants				
Sampling point	Influent flow / (m^3 d^{-1})	Population serviced	Treatment	Recipient river
STP 1	3801	7405	Oxidation pond	Langat River
STP 2	5721	24612	Oxidation ditch	Langat River
STP 3	1346	5984	Oxidation ditch	Langat River
STP 4	40000	180000	Oxidation ditch + anaerobic + aerobic	Linggi River
Effluent of hospitals				
Sampling point	No. beds	Population serviced	Treatment	Recipient river
HSP 1	850	3400	Discharge to STP 4	–
HSP 2	314	1256	Rotating biological contactor (RBC)	Muar River
HSP 3	93	372	Rotating biological contactor (RBC)	Ocean

SPE vacuum manifold (DOA-P504-BN, USA). Exactly 500 ng of the internal standard mixture, which was composed of caffeine-$^{13}C_3$ (CAF-$^{13}C_3$), simvastatin-D_6 (SMV-D_6), and diclofenac-D_4 (DIC-D_4), was added to the samples performed and mixed thoroughly before the extraction was performed. The solid-phase adsorbent was pre-conditioned with 2 mL of methyl *t*-butyl ether (MTBE), 2 mL of MeOH, and 2 mL of deionized water before the samples were loaded in the SPE cartridges. The samples were loaded at a flow rate of 9 mL min^{-1} under vacuum conditions. After sample loading, the solid-phase adsorbent was washed with 2 mL of DIW. The cartridges were then dried under vacuum at 15 mL min^{-1} for 25 min to 30 min to remove the residual water. The pharmaceutical compounds were subsequently eluted in 12 mL-glass tubes by sequentially passing 5 × 1 mL of MTBE, 2 × 1 mL of acetone-MeOH (21:9, v/v), and 3 × 1 mL of acetone-MeOH (9:21, v/v). The combined eluents were evaporated to dryness under a gentle stream of N_2 gas. The dry extracts were reconstituted with 500 µL of MeOH-DIW (10:90, v/v) and transferred to a 250 µL-deactivated glass insert with polymer feet inserted in amber glass vial (Agilent Technologies, USA). Exactly 30 µL of the extract was automatically injected into LC-electrospray ionization (ESI)-TOF/MS system for analysis.

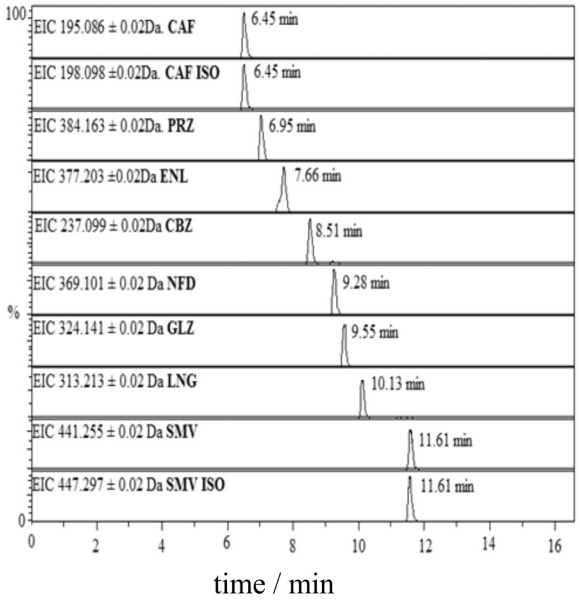

Figure 2. LC-ESI-TOF/MS extracted ionic chromatogram (EIC) showing the separation of 10 pharmaceutical compounds analysed in PI mode (100 µg L^{-1}).

LC-TOF/MS analysis

Instrumental analysis was carried out using a Dionex Ultimate 3000/LC 09115047 system (USA) equipped with a vacuum degasser, quaternary pump, and autosampler. The ESI interface consisted of the standard Z-spray™ ion source fitted with an electrospray probe.

The analytes that were detected in ESI (+) and ESI (–) modes were separated on the same column, as previously mentioned. The eight compounds, namely, caffeine, prazosin, enalapril, carbamazepine, nifedipine, gliclazide, levonorgestrel and simvastatin, and two internal standards that include CAF-$^{13}C_3$ and SMV-D_6 were analyzed in positive ion (PI) mode as shown in Figure 2. The three compounds, including hydrochlorothiazide, diclofenac-Na, and mefenamic acid, as well as the internal standard DIC-D_4 were analyzed in negative ion (NI) mode as presented in Figure 3.

Quality control

Recoveries of the studied pharmaceuticals in the different matrices of water samples were determined by initially spiking the samples with a standard mixture solution. The samples were then enriched using SPE. Six replicates were evaluated on different days, and the spiking levels used were 0.5, 1, 2, and 5 µg L^{-1} as shown in Table S1. Instrumental quantification limits are defined as the lowest concentration with a signal to noise ratio (S/N) of 10. Table S2 shows the linearity and limit of quantification (LOQ) of all the water samples.

Selectivity of the proposed method was investigated by analyzing the chromatograms obtained from the individual

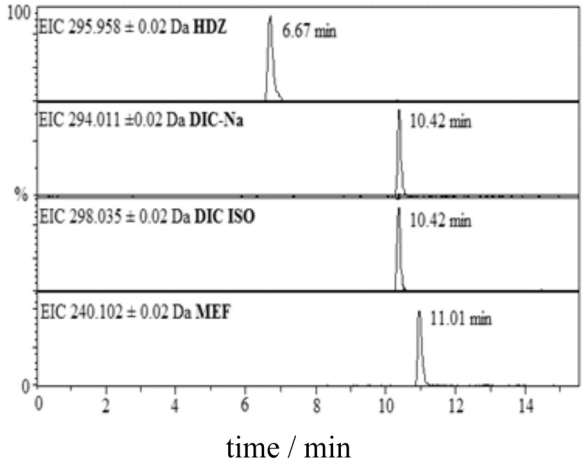

Figure 3. LC-ESI-TOF/MS extracted ionic chromatogram (EIC) showing the separation of 4 pharmaceutical compounds analysed in NI mode (100 μg L^{-1}).

standards, standard mixture, and solvent without standards. Robustness of the method was studied by changing the mobile phase flow, location of instrument, and volume of injection. The retention time was constant under all the conditions, indicating that the method was robust (Table S3).

Results and Discussion

Monitoring of pharmaceuticals in surface water, influent, and effluent of STP and HSPs

Occurrence of pharmaceuticals in sewage treatment plants

A total of 64 samples (i.e., influent and effluent of STPs) were collected and analysed by using LC-TOF/MS (Tables 3 and 4).

The frequency of detection and the concentration were 32:32 and 9099 ng L^{-1}, respectively; these values were expected for caffeine in influent of sewage treatment plants. The mean concentration of caffeine in the effluent of sewage treatment plants was 577 ng L^{-1}, which is considered low, compared with the results of other previous studies in the United Kingdom and Ireland at 2048 and 2700 ng L^{-1}, respectively.[28,29] Caffeine was moderately removed through the treatment process; hence, the high concentration might be related to irregular treatment timing, as observed in STP2. Consequently, the number of detection was 13:32 in the effluent of sewage treatment plants.

The maximum concentration of caffeine in the influent of the STPs was usually 9099 ng L^{-1}; however, the high concentration of caffeine detected in untreated wastewater was not only because of the amount of caffeine present in the pharmaceuticals, but also because of the presence of this compound in some products, such as coffee, tea, chocolate, and sports drinks.

The antihypertensive pharmaceuticals: prazosin, enalapril, nifedipine and hydrochlorothiazide were abundant in influent STP samples and had frequencies of detection of 18:32, 9:32, 1:32 and 27:32 respectively, and the maximum concentration was 525, 200, < LOQ and 287 ng L^{-1}, respectively. The poor detection of nifedipine may be related to the low consumption of consumers or rapid photodegradation when exposed to the environment. This result was in agreement with studies provided in Malaysia and Germany.[12,29] Three pharmaceuticals, namely carbamazepine, hydrochlorothiazide and gliclazide were the most persistent compounds after treatment with a maximum concentration of 344, 111 and 70 ng L^{-1}, respectively. In some cases, carbamazepine had higher effluent concentration than the STP influent. This finding has been noticed in other previous studies.[7,9,30] In this study, diclofenac-Na was detected in the influent and effluent of STP with a maximum concentration of 5049 ng L^{-1} and < LOQ, whereas mefenamic acid had maximum concentration of 296 and 30 ng L^{-1}.

In comparison, STP 4 caters for 25-fold more than STP1 and STP3. Furthermore, the pharmaceuticals detected over the same 8 months (May 2013 to December 2013) were found to be slightly low. This result might be attributed to the tertiary treatment conducted on the STP4.

Occurrence of pharmaceuticals in hospitals

A total of 24 samples were collected from 3 hospitals. Eight samples of the influent were collected from one hospital (HSP1), and 16 samples of the effluent were collected from the other two hospitals (HSP2 and HSP3) (Table 5 and 6). Table 5 shows the concentrations of studied pharmaceuticals in the influent of hospital HSP1. This hospital discharges directly to STP4; hence, no effluent sample was collected from this hospital. The concentration of pharmaceuticals in the influent HSP1 ranged from non-detected to 2979 ng L^{-1} as the maximum concentration. The highest concentration was for caffeine, that is, 2979 ng L^{-1} at high frequency detection 7:8. Antihypertensive pharmaceuticals, namely, prazosin, enalapril and hydrochlorothiazide, were detected at frequency detection of 3:8, whereas nifedipine was undetected in all samples. Levonorgestrel and diclofenac were detected only once in all collected samples. The concentration of pharmaceuticals in the effluent of hospital HSP2 and HSP3 are summarized in Table 6. The highest maximum concentration of 4131 ng L^{-1} was for caffeine, and the frequency of detection was 16:16. The occurrence of synthetic hormone (levonorgestrel) in the effluent of hospitals is presented in Table 6. However, the maximum and minimum concentrations of levonorgestrel were

Table 3. Mean, minimum, maximum and median concentrations (ng L^{-1}) for all studied pharmaceuticals in influent sewage treatment plants during eight months

Compound	May				June				July			
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4	STP 1	STP 2	STP 3	STP 4
CAF	2988	4000	4962	1911	1012	9099	7246	2321	2882	6256	2592	828
PRZ	107	14	327	157	57	97	161	213	< LOQ	26	ND	34
ENL	ND	ND	ND	ND	ND	ND	ND	< LOQ	ND	ND	33	11
CBZ	ND	47	ND	57	38	67	62	80	80	32	182	89
NFD	ND	< LOQ	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LNG	1267	98	707	ND	ND	ND	ND	ND	74	ND	91	166
SMV	ND	24	ND	35	20	27	27	ND	ND	25	ND	ND
HDZ	110	< LOQ	287	90	136	43	165	70	< LOQ	< LOQ	< LOQ	ND
GLZ	100	52	28	130	67	87	45	67	28	74	85	48
DIC	4043	ND	605	ND	279	ND	5049	ND	ND	< LOQ	ND	ND
MEF	ND	< LOQ	ND	207	ND	ND	ND	< LOQ	ND	< LOQ	< LOQ	ND
	August				September				October			
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4	STP 1	STP 2	STP 3	STP 4
CAF	5975	2217	4542	4309	4504	7771	7600	2232	3084	5469	2877	2389
PRZ	ND	ND	19	ND	ND	< LOQ	ND	ND	ND	88	28.3	ND
ENL	< LOQ	ND	ND	ND	ND	ND	ND	< LOD	ND	ND	ND	< LOD
CBZ	ND	ND	33	ND	ND	78	56	27	65	54	214	31
NFD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LNG	ND	72	ND	ND	ND	ND	31	ND	129	218	170	< LOQ
SMV	ND	20.3	ND	ND	ND	ND	ND	ND	ND	37	ND	35
HDZ	< LOQ	< LOQ	< LOQ	54	46	< LOQ	118	43	< LOD	< LOQ	< LOQ	55
GLZ	38	66	91	48	52	28	23	32	35	28	49	48
DIC	ND	ND	< LOQ	< LOQ	ND	ND	< LOD	ND	32	< LOQ	< LOQ	< LOQ
MEF	< LOQ	< LOQ	296	< LOQ	15	ND	< LOQ	< LOD	ND	47	ND	97
	November				December				Frequency	Mean	Min	Max	Median
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4					
CAF	590	797	677	3829	3865	4097	2742	2741	32:32	3700	590	9099	3036
PRZ	51	353	525	ND	ND	ND	ND	ND	18:32	141	14	525	92
ENL	ND	200	144	< LOD	ND	ND	ND	ND	9:32	97	11	200	88.5
CBZ	ND	250	156	42	36	29	45	37	25:32	76	27	250	56
NFD	ND	ND	ND	ND	ND	ND	ND	ND	1:32	ND	0	0	ND
LNG	ND	247	390	ND	ND	28	ND	ND	15:32	263	28	1267	147.5
SMV	19	ND	ND	20	ND	27	ND	ND	12:32	26	19	37	26
HDZ	ND	ND	ND	< LOQ	< LOD	44	56	< LOQ	27:32	94	43	287	63
GLZ	NM	NM	NM	36	22	68	67	44	29:29	55	22	130	48
DIC	ND	ND	ND	111	ND	ND	ND	ND	13:32	1687	32	5049	442
MEF	ND	ND	ND	225	ND	< LOQ	ND	83	17:32	139	15	296	97

CAF: caffeine; PRZ: prazosin; ENL: enalapril; CBZ: carbamazepine; NFD: nifedipine; LNG: levonorgestrel; SMV: simvastatin; HDZ: hydrochlorothiazide; GLZ: gliclazide; DIC: diclofenac-Na; MEF: mefenamic acid; ND: not detected; < LOQ: the lowest limit at which analyte could be quantified.

105 and 73 ng L^{-1}, respectively. The antidiabetic drug (gliclazide) was persistent in the effluent of hospitals and had a frequency of detection of 15:17 and a maximum concentration of 66 ng L^{-1}. Hydrochlorohtiaizde was the most frequent detected drug compared with other pharmaceuticals in similar therapeutic classes at a maximum concentration of 78 ng L^{-1}. Carbamazepine was found at a concentration range of 12 to 142 ng L^{-1} and the frequency of detection was 12:18. Nifedipine was detected only once at a concentration of 264 ng L^{-1}. The efficiency of treatment for sewage treatment plants compared with hospitals is considered better in terms of the number of detection and concentration of pharmaceuticals.

Occurrence of pharmaceuticals in surface water

A total of 15 samples were collected from two rivers (Table 7). Caffeine is one of the most widely used nervous system drugs (stimulant drug) and is available over the counter. Caffeine was continuously detected in the surface water at a high frequency number of detection (15:15)

Table 4. Mean, minimum, maximum and median concentrations (ng L^{-1}) for all studied pharmaceuticals in effluent sewage treatment plants during eight months

Compound	May				June				July			
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4	STP 1	STP 2	STP 3	STP 4
CAF	ND	1464	ND	ND	ND	< LOQ	ND	< LOQ	149	ND	ND	< LOQ
PRZ	ND	ND	ND	ND	77	ND	ND	16	7	ND	ND	14
ENL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9
CBZ	19	17	28	38	13	15	19	18	ND	54	51	46
NFD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LNG	ND	ND	ND	ND	ND	ND	ND	ND	16	ND	< LOQ	35
SMV	10	22	11	ND	ND	8.3	8.3	8	8	16	ND	7
HDZ	< LOQ	46	53	111	19	48	52	< LOQ	6	62	26	ND
GLZ	18	39	16	51	13	51	16	70	34	36	17	34
DIC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEF	ND	ND	ND	ND	ND	ND	ND	< LOQ	ND	< LOQ	ND	ND
	August				September				October			
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4	STP 1	STP 2	STP 3	STP 4
CAF	ND	ND	ND	117	ND	ND	ND	ND	ND	< LOQ	< LOQ	< LOQ
PRZ	ND	13	7	ND	8	ND	ND	14	ND	11	15	ND
ENL	ND	ND	ND	ND	ND	2	ND	ND	ND	ND	ND	ND
CBZ	20	16	344	31	16	16	40	36	11	21	123	40
NFD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LNG	ND	36	26.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
SMV	ND	14	12	ND	ND	ND	8	ND	ND	ND	ND	ND
HDZ	ND	28	16	69	< LOD	31	< LOQ	< LOQ	ND	25	< LOQ	60
GLZ	23	33	24	34	13	12	16	32	9	18	12	29
DIC	ND	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND	ND	ND	ND	ND
MEF	ND	30	13	17	6	5	< LOD	5	ND	6	ND	15
	November				December				Frequency	Mean	Min	Max	Median
	STP 1	STP 2	STP 3	STP 4	STP 1	STP 2	STP 3	STP4					
CAF	ND	< LOQ	< LOQ	ND	< LOQ	ND	ND	< LOQ	13:32	577	117	1464	149
PRZ	ND	116	< LOQ	< LOQ	ND	ND	ND	ND	13:32	27	7	116	14
ENL	ND	62	ND	ND	ND	ND	ND	ND	3:32	24	2	62	9
CBZ	18	45	16	ND	17	12	27	32	030:32	40	11	344	20.5
NFD	ND	ND	ND	ND	ND	ND	ND	ND	0:32	ND	0	0	ND
LNG	ND	66	ND	ND	ND	ND	ND	ND	006:32	36	16	66	35
SMV	ND	ND	ND	ND	ND	ND	ND	ND	012:32	11	7	22	9.2
HDZ	ND	ND	< LOQ	32	ND	27	29	62	026:32	42	6	111	32
GLZ	NM	NM	NM	14	25	13	16	32	029:29	26	9	70	23
DIC	ND	ND	ND	ND	ND	ND	ND	ND	3:32	ND	0	0	ND
MEF	ND	ND	< LOQ	12	ND	18	< LOQ	21	016:32	14	5	30	13

CAF: caffeine; PRZ: prazosin; ENL: enalapril; CBZ: carbamazepine; NFD: nifedipine; LNG: levonorgestrel; SMV: simvastatin; HDZ: hydrochlorothiazide; GLZ: gliclazide; DIC: diclofenac-Na; MEF: mefenamic acid; ND: not detected; < LOQ: the lowest limit at which analyte could be quantified.

with the highest concentration of 1213 ng L^{-1}. This result might be due to the non-prescribed property of caffeine. However, caffeine was detected in surface water in Italy and Romania, with maximum concentration of 1056 and 3480 ng L^{-1}, respectively.[31,32] The lowest concentration of caffeine detected in surface water was 116 ng L^{-1}; this result was in agreement with our previous study, wherein the concentration of caffeine was 257 ng L^{-1}.[10]

Antihypertensive pharmaceuticals, prazosin, enalapril, nifedipine and hydrochlorothiazide, were detected at varying frequencies. However, nifedipine was undetected in surface water during all periods of the study. This phenomenon may be related to the fact that nifedipine is easily degraded and light sensitive. Hence, nifedipine did not show high persistency in the environment. Nifedipine was also seldom reported in the environment and was absent in 11 rivers and streams samples collected from Germany.[12] This was the first time that prazosin was reported in environment matrices and was found at a concentration of 2 to 30 ng L^{-1}. The

Table 5. Mean, minimum, maximum and median concentrations (ng L^{-1}) for all studied pharmaceuticals in influent hospitals during nine months

Compound	April	May	June	July	August	September	October	November	December	Frequency	Mean	Min	Max	Median
	HSP 1	HSP 1	HSP 1	HSP 1	HSP 1	HSP 1	HSP 1	HSP 1	HSP 1					
CAF	2979	2802	964	ND	NM	2707	482	400	1021	7:08	1419	400	2979	1021
PRZ	ND	119	< LOQ	ND	NM	ND	ND	120	ND	3:08	119	119	120	119.5
ENL	63	ND	ND	ND	NM	ND	ND	352	28	3:08	148	28	352	63
CBZ	ND	ND	ND	ND	NM	ND	27	687	39	3:08	188	27	687	39
NFD	ND	ND	ND	ND	NM	ND	ND	ND	ND	0:08	ND	ND	ND	ND
LNG	ND	ND	ND	ND	NM	ND	ND	608	ND	1:08	608	608	608	608
SMV	71	31	28	30	NM	ND	36	ND	ND	5:08	39	28	71	31
HDZ	ND	50	ND	ND	NM	49	104	ND	ND	3:08	68	49	104	50
GLZ	ND	ND	ND	27	NM	19	Nd	28	21	4:08	19	19	28	24
DIC	ND	ND	ND	ND	NM	ND	< LOQ	ND	ND	1:08	ND	ND	ND	ND
MEF	ND	ND	ND	< LOQ	NM	ND	74	ND	ND	2:08	74	74	74	74

CAF: caffeine; PRZ: prazosin; ENL: enalapril; CBZ: carbamazepine; NFD: nifedipine; LNG: levonorgestrel; SMV: simvastatin; HDZ: hydrochlorothiazide; GLZ: gliclazide; DIC: diclofenac-Na; MEF: mefenamic acid; ND: not detected; < LOQ: the lowest limit at which analyte could be quantified.

Table 6. Mean, minimum, maximum and median concentrations (ng L^{-1}) for all studied pharmaceuticals in effluent hospitals during nine months

Compound	April		May		June		July		August		September	
	HSP 2	HSP 3	HSP 2	HSP 3	HSP 2	HSP 3	HSP 2	HSP 3	HSP 2	HSP 3	HSP 2	HSP 3
CAF	574	3437	574	2194	41	2426	682	2526	450	4131	136	2269
PRZ	13	ND	13	ND	6	69	ND	ND	ND	ND	19	ND
ENL	40	88	40	6	3	ND	ND	ND	ND	ND	ND	ND
CBZ	14	ND	14	32	12	ND	23	ND	25	ND	142	ND
NFD	ND	264	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LNG	ND	ND	ND	ND	ND	73	ND	ND	ND	ND	ND	ND
SMV	8	ND	8	63.2	ND	ND	ND	ND	ND	ND	ND	19
HDZ	23	78	23	72	31	48	47	52	74	25	33	32
GLZ	Nd	30	25	26	8	66	13	20	19	9	20	14
DIC	60	ND	59	ND	32	115	51	21	88	ND	15	ND
MEF	31	ND	< LOQ	< LOQ	< LOQ	284	28	85	50	ND	< LOQ	193

	October		November		December		Frequency	Mean	Min	Max	Median
	HSP 2	HSP 3	HSP 2	HSP 3	HSP 2	HSP 3					
CAF	545	2043	174	1916	363	1937	18:18	1468	41	4131	1299
PRZ	112	ND	117	ND	10	14	9:18	41	6	117	14
ENL	ND	ND	51	< LOQ	ND	ND	7:18	38	3	88	40
CBZ	ND	16	81	18	44	15	12:18	36	12	142	20.5
NFD	ND	ND	ND	ND	ND	ND	1:18	264	264	264	264
LNG	ND	< LOQ	105	ND	ND	ND	3:18	89	73	105	89
SMV	< LOQ	14	8	ND	ND	10	8:18	19	8	63	10
HDZ	15	45	ND	32	55	ND	16:18	43	15	78	39
GLZ	12	20	NM	12	16	ND	15:17	21	8	66	19
DIC	43	15	ND	ND	ND	ND	10:18	50	15	115	47
MEF	23	< LOQ	ND	< LOQ	< LOQ	ND	14:18	99	23	284	50

CAF: caffeine; PRZ: prazosin; ENL: enalapril; CBZ: carbamazepine; NFD: nifedipine; LNG: levonorgestrel; SMV: simvastatin; HDZ: hydrochlorothiazide; GLZ: gliclazide; DIC: diclofenac-Na; MEF: mefenamic acid; ND: not detected; < LOQ: the lowest limit at which analyte could be quantified.

number of frequency detection of prazosin and enalapril was 6:15 and 3:15, respectively. This result might be due to the rapid metabolization of enalapril by liver esterases to enalaprilat. However, a few previous studies reported that enalapril was undetected in surface water or < LOQ.[33,34] The maximum concentration of enalpril was 14 ng L^{-1}. Hydrochlorothiazide is a diuretic drug and is the most detected pharmaceutical (13:15) in this group. The maximum and lowest concentrations were 54 and 4 ng L^{-1}, respectively. Hydrochlorothiazide was detected in Spain, at varying concentrations 164 and 8 ng L^{-1}.[33,35] Simvastatin is the methylated form of

lovastatin, is used to treat primary hypercholesterolemia, and is effective in reducing total and low-density lipoprotein (LDL)-cholesterol. Simvastatin was detected in surface water at low concentrations of 6 ng L^{-1}, as a maximum concentration with a poor frequency of detection 5:15. This result was in line with previous study reported by Al-Odaini,[29] who detected simvastatin in Malaysia at concentration less than method detection limit (MDL). Carbamazepine, diclofenac, mefenamic acid, and gliclazide, that belong to different therapeutic classes, have been detected at different concentrations of 53, 54, 40 and 36 ng L^{-1}, respectively (Table 7). The anti-diabetic drug, gliclazide, was detected at a high frequency number of 12:13. This observation might be attributed to 8% of the population in Malaysia take this drug.[20] The impact of pharmaceuticals detected in surface water varied from sewage treatment plants to hospital discharge. However, most pharmaceuticals detected in SW HSP2 have concentration higher than those detected in SW STP1. This result could be attributed to the efficiency of treatment process in sewage treatment plants and hospitals.

Chemometric analysis

Chemometric analysis results showed that the water sampling stations were classified into three clusters, as shown in the dendogram (Figure 4).

The first cluster included the influents STP 1, STP 2, STP 3, STP 4 and the HSP 3, whereas the HSP 1 forms the second cluster. The effluents STP 1, STP 2, STP 3, and STP 4, HSP 2, downstream river of STP 1, and downstream river hospital HSP 2 represent the third cluster. The effluents from the two hospitals (i.e., HSP 2 and HSP 3) formed different clusters because of the various consumed pharmaceuticals and treatment process efficiency in each hospital. The similarity between sampling stations in the first cluster may be attributed to the similarity of the type of sample in terms of a highly polluted sample (e.g., sewage treatment plants). However, the effluent of the hospital HSP 3 was included in the first cluster because of the frequency of detection of most compounds in this location. The influent of the hospital HSP 1 was clustered alone (second cluster) because of the

Table 7. Mean, minimum, maximum and median concentrations (ng L^{-1}) for all studied pharmaceuticals in surface water during eight months

Compound	May		June		July		August		September		October	
	SW STP1	SW HSP2	SW STP1	SW HSP2	SW STP1	SW HSP2	SW STP1	SW HSP2	SW STP1	SW HSP2	SW STP1	SW HSP2
CAF	138	360	760	389	463	353	417	343	1213	NM	164	318
PRZ	6	18	30	10	2	ND	ND	ND	ND	NM	ND	ND
ENL	ND	14	ND	7	ND	ND	ND	ND	ND	NM	ND	ND
CBZ	10	14	ND	24	ND	11	6	11.4	7	NM	ND	53
NFD	ND	ND	ND	ND	ND	ND	ND	ND	ND	NM	ND	ND
LNG	ND	ND	ND	ND	ND	ND	ND	ND	< LOQ	NM	< LOQ	12
SMV	ND	ND	5	ND	6	ND	ND	ND	ND	NM	4	6
HDZ	17	33	14	54	17	49	4	31	< LOQ	NM	9	33
GLZ	Nd	36	7	17	22	8	6	8	6	NM	5	10
DIC	ND	54	ND	52	9	ND	< LOQ	18	ND	NM	ND	54
MEF	6	17	ND	38	10	3	5	12	ND	NM	4	40

	November		December		Frequency	Mean	Min	Max	Median
	SW STP1	SW HSP2	SW STP1	SW HSP2					
CAF	116	139	760	890	15:15	427	116	1213	360
PRZ	ND	28	ND	ND	6:15	16	2	30	14
ENL	ND	13	ND	ND	3:15	11	7	14	13
CBZ	13	25	ND	14	11:15	16	6	53	13
NFD	ND	ND	ND	ND	0:15	ND	ND	ND	ND
LNG	ND	22	ND	ND	4:15	17	12	22	17
SMV	ND	4	ND	ND	5:15	5	4	6	5
HDZ	ND	ND	17	39	13:15	24	4	54	24
GLZ	NM	NM	10	13	12:13	11	5	36	9
DIC	ND	ND	ND	21	7:15	35	9	54	36.5
MEF	ND	ND	ND	11	10:15	15	3	40	10.5

CAF: caffeine; PRZ: prazosin; ENL: enalapril; CBZ: carbamazepine; NFD: nifedipine; LNG: levonorgestrel; SMV: simvastatin; HDZ: hydrochlorothiazide; GLZ: gliclazide; DIC: diclofenac-Na; MEF: mefenamic acid; ND: not detected; NM: not measured; < LOQ: the lowest limit at which analyte could be quantified; SW STP1: surface water downstream effluent STP1; SW HSP 2: surface water downstream effluent of hospital HSP 2.

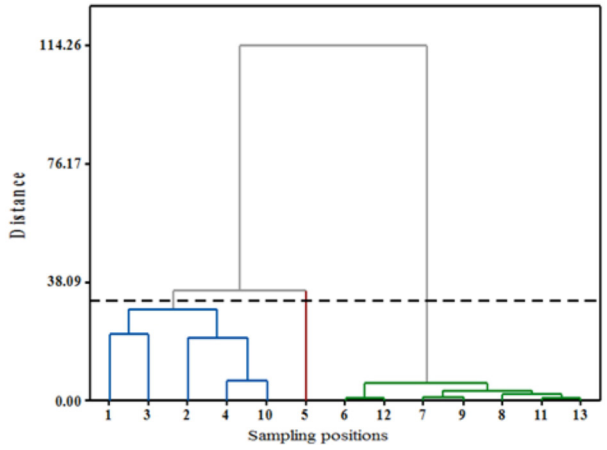

Figure 4. Dendogram showing different clusters of different sampling stations of water samples on pharmaceutical pollution. 1: INF STP 1; 2: INF STP 2; 3: INF STP 3; 4: INF STP 4; 5: INF HSP 1; 6: EFF STP 1; 7: EFF STP 2; 8: EFF STP 3; 9: EFF STP 4; 10: EFF HSP 3; 11: EFF HSP 2; 12: SW STP 1; 13: SW HSP 2.

design of the sampling point, which is as pipe discharge direct to the sewage treatment plant STP 4, it was not sink like other stations as common. The other sampling points, including the effluent of the STPs, effluent of the hospital HSP 2, and their related downstream river, was considered as cluster three. These locations were grouped together because of the high similarity in terms of concentration and frequency of detection in these locations.

Figure 5 shows the dendogram cluster of the pharmaceuticals, in which three clusters were formed. All studied compounds were clustered in different groups because they do not form one cluster. These differences were attributed to the differences in the physicochemical properties, environmental fate, and ability of the pharmaceuticals to resist degradation.

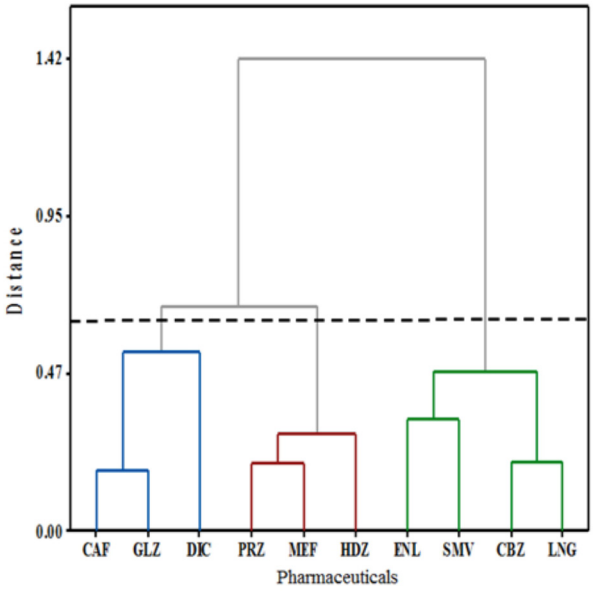

Figure 5. Dendogram showing different clusters of pharmaceuticals detected at different water samples.

Caffeine, gliclazide, and diclofenac-Na comprised the first cluster. The second cluster included prazosin, mefenamic acid, and hydrochlorothiazide, whereas enalapril, simvastatin, carbamazepine, and levonorgestrel constituted the third cluster. The studied pharmaceuticals were clustered based on their environmental behaviour or properties. The grouping of caffeine, gliclazide, and diclofenac-Na may be due to their high consumption and non-prescribed pharmaceuticals (caffeine).

The principal component analysis was used to select the number of components extracted. According to the eigenvalue-one criterion, only principal components (PCs) with eigenvalues greater than 1 are considered as important values. In the scree plot test (Figure 6), three PCs were selected, in which the eigenvalues for PC1, PC2, and PC3 were 5.756, 1.5877, and 1.091, respectively. The scores of the samples corresponding to PC1 and PC2 are presented in Figure 7. Each sample was identified by the name of the corresponding sampling station. The sample sites were classified in three different groups. The first group corresponds to the zone with high concentration influence, which included the influent of the four STPs (i.e., INF STP 1, INF STP 2, INF STP 3, and INF STP 4) and the effluent of the HSP 2. The second group corresponds to the influent of the hospital (INF HSP 1). Meanwhile, the third group includes the effluent of the STPs, effluent of the HSP 3, and their downstream river (i.e., SW STP 1 and SW HSP 2), which considered less pollution compared with zones 1 and 2.

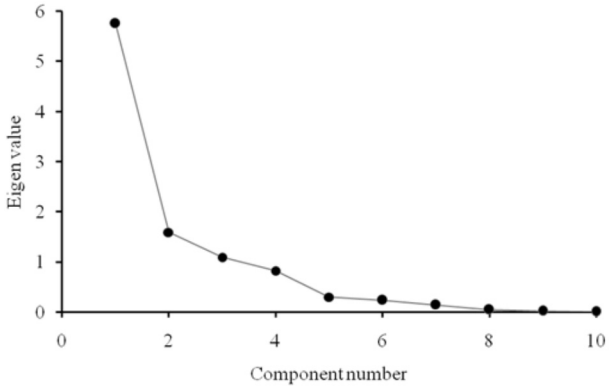

Figure 6. Scree plot of the eigenvalues of principal components.

Table 8 shows the loading of varimax-rotated factor matrix for three-factor models. Evidently, the first factor was generally more correlated with the variables than the second and the third factors. The terms ‘strong’, ‘moderate’, and ‘weak’ as applied to factor loadings, refer to absolute loading values of > 0.75, 0.75-0.5, and 0.5-0.3, respectively.

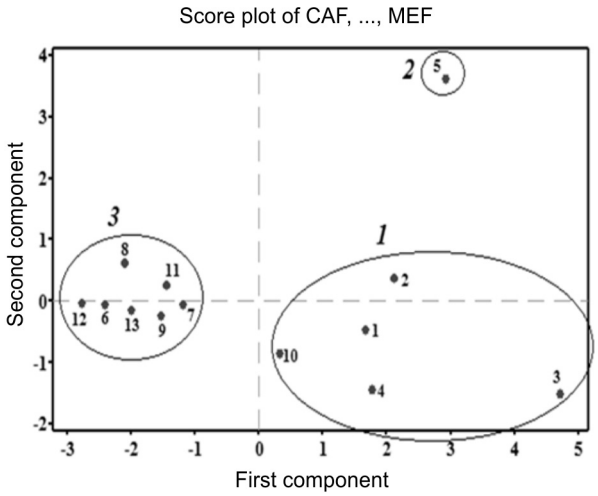

Figure 7. Scores of all 13 water samples on the plane defined by the first two principal components.

Factor 1 (VF1), which contributed 57.6% of the total variability (Table 8), was strong-positive correlated to caffeine, whereas prazosin, gliclazide, and mefenamic acid were related to clusters 1 and 2.

Factor 2 (VF2) explains 15.9% of the data variability, with a clear contribution from enalapril, carbamazepine, levonorgestrel, and simvastatin; these compounds were included entirely in cluster 3. Factor 3 (VF3) explains 10.9% of the variability. However, only two compounds were included in the VF3, namely diclofenac-Na and hydrochlorothiazide, which belong to clusters 1 and 2, respectively.

Overall, the 10 pharmaceuticals detected in the bodies of water in Malaysia were distributed in the influent and effluent of the STPs and HSPs, as well as in the SW.

Table 8. Rotated factor loadings and communalities varimax rotation

Compound	VF1	VF2	VF3
Caffeine	0.850	0.293	–0.16
Prazosin	0.833	0.268	–0.305
Enalapril	0.44	0.776	0.203
Carbamazepine	0.054	0.944	–0.198
Gliclazide	0.828	0.111	–0.248
Levonorgestrel	0.074	0.755	–0.608
Simvastatin	0.626	0.680	–0.098
Hydrochlorothiazide	0.509	0.356	–0.612
Diclofenac-Na	0.342	–0.008	–0.879
Mefenamic acid	0.845	0.059	–0.191
Eigen value	5.7568	1.5877	1.0919
Variability / %	57.6	15.9	10.9
Cumulative / %	57.6	73.4	84.4

Conclusions

A methodology using LC-TOF/MS analysis of caffeine, prazosin, enalapril, carbamazepine, nifedipine, levonorgestrel, simvastatin, hydrochlorothiazide, gliclazide, diclofenac-Na, and mefenamic acid was developed and successfully applied to analyze the different environmental aquatic samples in the SW, and influent and effluent of the STPs and HSPs. These pharmaceuticals were chosen based on their reported high consumption rates in Malaysia and frequent detection worldwide. The proposed method was precise, sensitive, and robust. Moreover, the method was accurate and the method facilitated the detection of 11 compounds from the water samples. Accurate mass measurements were monitored for each of the pharmaceuticals, which were studied using TOF/MS. The LOQs varied broadly, depending on the compound; the values of the LOQ ranged from 0.4 ng L^{-1} to 3 ng L^{-1}, 1.6 ng L^{-1} to 13 ng L^{-1}, 2.2 ng L^{-1} to 46 ng L^{-1}, 2.8 ng L^{-1} to 28 ng L^{-1}, 11 ng L^{-1} to 182 ng L^{-1}, and 5 ng L^{-1} to 267 ng L^{-1} in drinking water, SW, sewage treatment plants and hospital effluents, and in sewage treatment plants and hospital influent, respectively. The current work investigated the presence of the eleven pharmaceuticals in the SW and influent and effluent of the sewage treatment plants and hospitals in Malaysia.

The results showed that a number of the studied compounds namely; caffeine, carbamazepine, gliclazide, simvastatin, hydrochlorothiazide and mefenamic acid; pose moderate to high persistence in sewage treatment effluents, as well as in the receiving rivers. The compounds detected in the 105 samples at all sampling points during the nine-month monitoring from April 2013 to December 2013 were as follows: 81.5% caffeine, 43% prazosin, 18.5% enalapril, 75% carbamazepine, 92.7% gliclazide, 28.7% levonorgestrel, 41.7% simvastatin, 86.1% hydrochlorothiazide, 37% diclofenac-Na, and 61.1% mefenamic acid. Nifedipine was detected in only one of the 105 tested samples.

This study confirmed that the bodies of water in Malaysia contained varying levels of different pharmaceutical residues.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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