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Research Article

Licit and Illicit Drugs in Urban Wastewater in Cyprus

Two multi-residue methods based on off-line solid phase extraction followed by LC-MS/MS have been applied in samples collected from three sewage treatment plants for the identification and quantification of licit and illicit drugs. The occurrence of the drugs in wastewater appears to be influenced by both the operational parameters of each plant and their consumption in the area served by the corresponding plant. Generally, the present study demonstrated that both licit and illicit drugs were found in the effluent wastewater of all urban wastewater treatment plants; a fact suggesting that they are bioresistance. These compounds were determined in relatively high concentrations (licit drugs: below limit of detection (BLD) to 5520 ng/L and illicit drugs: BLD to 5815 ng/L) in all effluent wastewater samples monitored in this study. The major groups of licit drugs detected in the effluents were antibiotics, β-blockers, analgesics, and non-steroidal-anti-inflammatory drugs suggesting that these compounds show a noticeable resistance against the biological treatment applied. The concentrations of antibiotics and β-blockers in the effluents ranged from ca. 4 to 200 ng/L and 40 to 500 ng/L, respectively. Concerning the illicit drugs, cocaine and its metabolite benzoylecgonine were detected in almost all effluent samples with median values of 1 and 3 ng/L, respectively.

Keywords: LC-MS/MS; Micropollutants; Sewage treatment plants; Solid phase extraction; Wastewater treatment

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1 Introduction

Several studies during the last two decades have indicated that many organic micropollutants including licit and illicit drugs are not completely removed at urban wastewater treatment plants (UWTPs), entering thus the ecosystem through the treated effluent [1, 2]. In recent years, there is an increasingly growing momentum towards the reuse of wastewater while at the same time the concern with respect to the existence of xenobiotic compounds including drug residues in the effluent wastewater (EWW) also increases. These compounds are persistent against biological degradation at the treatment plants and they may enter into the aquatic environment such as ground water [3], surface water [4], as well as onto sludge, soil and sediments [5], and therefore they may remain in the environment for a long time.

Many relevant studies have proved that UWTPs do not completely eliminate drugs such as non-steroidal-anti-inflammatory drugs (NSAIDs), antibiotics, β-blockers, etc., and their presence has been confirmed in effluent wastewater (EWW) in various countries worldwide at concentrations ranging from few ng/L to μg/L [6–9].

Several analytical methods such as liquid chromatography (LC) tandem mass spectrometry (MS) (LC-MS/MS) are available for the determination of such compounds in different aquatic matrices [2, 4]. Chromatographic methods have been applied to wastewater samples, in order to evaluate the removal efficiency [10] and/or their existence, and most recently to raw wastewater in the framework of epidemiological studies to estimate the illicit drug use in communities [11].

Illicit drugs are one of the latest groups of organic substances that have received significant attention from scientists since only recently were recognized as pollutants of emerging concerns, present in the ecosystem [4]. Some of these drugs are found to be quite persistent against wastewater treatment, remaining for a long time in the environment. According to various studies, influent wastewater samples (IWWs) in Belgium, Germany, Italy, Spain, Switzerland, contain a variety of illicit drugs like cocaine,

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Abbreviations: BLD, below limit of detection; **BLQ**, below limit of quantification; **EWW**, effluent wastewater; **HPLC**, high performance liquid chromatography; **IWW**, influent wastewater; **LC-MS/MS**, liquid chromatography tandem mass spectrometry; **LOD**, limit of detection; **LOQ**, limit of quantification; **LSD**, lysergic acid diethylamide; **MDA**, 3,4-methylenedioxyamphetamine; **MDEA**, 3,4-methylenedioxyethamphetamine; **MDMA**, 3,4-methylenedioxyethamphetamine or ecstasy; **MEC**, measured environmental concentration; **NSAID**, non-steroidal-anti-inflammatory drug; **PEC**, predicted environmental concentration; **SPE**, solid phase extraction; **SWW**, secondary wastewater; **UPLC-MS/MS**, ultra-performance liquid chromatography tandem mass spectrometry; **UWTP**, urban wastewater treatment plant

benzoylecgonine, morphine, opioid drugs, amphetamine, and others with concentrations between below the limit of quantification (LOQ) and 1700 ng/L [12–15]. For the quantification of illicit drugs and their metabolites in the aquatic environment, especially in wastewater, solid phase extraction (SPE) followed by LC-MS/MS methods have been developed [11–13].

The present study describes the application of two fast and accurate multi-residue methods for investigating the occurrence of multi-class licit and illicit drugs in wastewater samples, using off-line SPE followed by LC-MS/MS. The main objectives of this study were: (i) to apply a sensitive method for trace analysis of 29 licit and 26 illicit drugs and their metabolites at various stages of the treatment process at three and two UWTPs in Cyprus, respectively; and (ii) to evaluate the removal efficiency of the UWTPs with respect to licit and illicit drugs. This paper is the first report on the occurrence of illicit drugs and their metabolites in UWTPs in Cyprus, and the second most extended report on licit drugs [16].

According to the authors' knowledge, this work is also the first one revealing data and information on the presence of illicit drugs in UWTP influents and effluents for a Mediterranean island, which potentially presents a different case study compared to other mainland countries. Being a highly water-stressed country, Cyprus is directing intense efforts to wastewater reuse applications (i.e., for irrigation and groundwater replenishment). Therefore, knowledge on the existing situation with regard to the presence of licit and illicit drugs in the treated effluents is of particular interest.

2 Materials and methods

2.1 Chemicals

The licit drugs (Table 1) were purchased from J. Escuder (Rubí, Spain), Sigma–Aldrich (Steinheim, Germany), and LGC Promochem (London, UK). Isotopically labeled compounds, used as internal standards, were ^{13}C-phenacetin obtained from Sigma–Aldrich, mecoprop-d_3 from Dr. Ehrenstorfer (Augsburg, Germany), ibuprofen-d_3, atenolol-d_7, and carbamazepine-d_{10} from CDN Isotopes (Quebec, Canada). The illicit drugs (Table 2) and surrogate labeled standards (morphine-d_6, codeine-d_6, 6-acetylmorphine-d_6, heroin-d_9, fentanyl-d_5, EDDP-d_3, methadone-d_9 were purchased from Cerilliant (Austin, TX, USA).

Table 1. Licit drugs' concentrations in IWW, SWW, and EWW samples for three UWTPs

	Concentration of licit drugs (ng/L)								
	IWW			SWW			EWW		
Compound	Occ.	Mean	Range	Occ.	Mean	Range	Occ.	Mean	Range
NSAIDs									
Acetaminophen	6/6	115 004	7871–232 300	3/6	510	BLD–700	3/6	553	BLD–650
Diclofenac	6/6	2339	1202–3457	5/6	1828	506–3283	3/6	804	BLD–2950
Ibuprofen	6/6	2801	1680–5315	6/6	58	40–103	5/6	43	BLD–82
Indomethacine	6/6	73	19–120	6/6	69	36–120	2/6	23	BLD–110
Ketoprofen	3/6	900	BLD–956	3/6	162	BLD–273	2/6	89	BLD–120
Mefenamic acid	6/6	1411	1040–2040	6/6	493	183–814	4/6	68	BLD–310
Naproxen	3/6	90	BLD–210	1/6	10	BLD–10	0/6	BLD	BLD
Propyphenazone	1/6	10	BLD–10	4/6	3	BLD–12	0/6	BLD	BLD
Lipid regulators and cholesterol lowering statin drugs									
Bezafibrate	6/6	1104	519–1610	6/6	205	88–310	6/6	108	25–170
Clofibric acid	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Gemfibrozil	6/6	476	71–1080	6/6	465	70–970	4/6	139	BLD–480
Mevastatin	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Pravastatin	6/6	70	23–130	4/6	27	BLD–40	1/6	8	BLDk8
Psychiatric drugs									
Carbamazepine	6/6	1720	460–4023	6/6	2082	510–5811	5/6	1820	BQL–5520
Fluoxetine	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Paroxetine	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Anti-ulcer agent									
Lansoprazole	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Histamine H1 and H2 receptor antagonistics									
Famotidine	6/6	1181	490–2320	5/6	555	BLD–1740	1/6	20	BLD–20
Loratadine	0/6	BLD	BLD	0/6	BLD	BLD	0/6	BLD	BLD
Ranitidine	6/6	207	28–640	5/6	99	BLD–260	1/6	30	BLD–30
Antibiotics									
Azythromycin	0/6	BLD	BLD	4/6	71	BLD–210	2/6	38	BLD–70
Erythromycin	5/6	97	BLD–428	6/6	66	10–217	3/6	32	BLD–140
Ofloxacin	6/6	615	220–2360	6/6	980	130–4726	5/6	218	BLD–353
Sulfamethoxazole	6/6	305	178–350	6/6	100	BLQ–320	4/6	76	BLD–290
Trimethoprim	6/6	71	30–130	5/6	17	BLD–30	2/6	4	BLQ–4
β-Blockers									
Atenolol	6/6	3733	2750–4740	6/6	950	120–15 940	6/6	466	10–900
Metoprolol	6/6	340	196–610	6/6	186	126–250	6/6	92	20–210
Propranolol	5/6	45	BLD–270	6/6	73	31–170	3/6	40	BLD–90
Sotalolol	6/6	597	336–950	6/6	404	310–590	4/6	121	BLD–330

BLD ($LOD_{influent}$ = 3 ng/L, $LOD_{effluent}$ = 1 ng/L); BLQ ($LOQ_{influent}$ = 6 ng/L, $LOQ_{efflunet}$ = 3 ng/L).
Occ., occurrence; number of samples in which the compounds were detected/number of samples examined.

Methanol, acetonitrile, and water (LiChrosolv) were supplied by Merck (Darmstadt, Germany). Hydrochloric acid 37%, NH_4Ac and HAc were obtained from Merck and Sigma–Aldrich (MO, USA). Nitrogen for drying of 99.995% purity was purchased from Air Liquide (Spain).

A stock solution of each compound was prepared in methanol and stored at −20°C.

2.2 Sampling

Wastewater samples were collected from three UWTPs (UWTP I, II, and III) located in the south of Cyprus, whose treatment consist of pretreatment, preliminary treatment, primary sedimentation, secondary (biological) treatment, sand filtration, and chlorination. The pretreatment process includes a physical process of settling in a primary clarifier. The secondary treatment consists of pre-nitrification (anaerobic) and nitrification (aerobic) tanks, and (i) oxidation ditches at UWTP I, (ii) conventional activated sludge including aeration tanks at UWTP II, and (iii) two secondary clarifiers at UWTP III. It is important to note that these treatment plants were selected for this study since they serve the largest areas on the island.

For the determination of the licit drugs, six influent wastewater samples (IWW, after primary clarifier), six secondary wastewater samples (SWW, after secondary clarifier), and six effluent wastewater samples (EWW, after sand filter) were collected from the three UWTPs. The wastewater samples were collected twice, in spring (May) and summer (August). Three stages of the treatment have been examined in order to potentially understand more thoroughly the removal capacity of the various processes.

For the study of the occurrence of illicit drugs (and of the licit compounds caffeine, cotinine, nicotine, and paraxanthine), eight IWW and EWW samples were collected from UWTP I and II, which are the two biggest plants. The sampling was performed during four consecutive weeks, within one month (i.e., November).

Daily-composite samples were obtained by mixing sample volumes collected during 24 h using a time-proportional automatic sampler (ISCO 6712). All samples were stored at 4°C until analysis. During sampling, acidification of the samples was done onsite before transporting (within 1 h) to the laboratory. The time between sampling and analysis was less than seven days. The main operational characteristics of the UWTPs are presented in Supporting Information Table S1.

2.3 Analytical methodology

The samples were filtered through 2.7, 1 μm glass fiber filters and 0.45 μm nylon membrane filters (Whatman, Maidstone, Kent, UK). All samples were enriched by SPE, using OASIS-HLB cartridges (60 mg, 3 mL, Waters, Milford, MA, USA). The procedure of SPE is described in detail elsewhere [6, 12].

The determination of licit and illicit drugs and their metabolites in wastewater samples using off-line SPE followed by LC-MS/MS was

Table 2. Concentrations of illicit drugs, caffeine, cotinine, nicotine, and paraxanthine in IWW and EWW samples

	Concentration (ng/L)					
	IWW			EWW		
Compound	Occ.	Mean	Range	Occ.	Mean	Range
6-Acetylmorphine	1/8	BLQ	BLQ	1/8	BLQ	BLQ
Amphetamine	7/8	BLQ	BLQ	0/8	BLD	BLD
Benzoylecgonine	8/8	37	13–53	8/8	3	1–7
Caffeine	8/8	13 964	21–15 834	8/8	1859	293–5815
Cocaethylene	8/8	BLQ	BLQ	0/8	BLD	BLD
Cocaine	8/8	3	BLQ–8	8/8	1	BLQ–1
Codeine	8/8	4384	2316–6460	7/8	1864	BLQ–3783
Cotinine	8/8	1024	631–1356	8/8	15	3–25
EDDP	7/8	BLQ	BLQ	8/8	BLQ	BLQ
Fentanyl	0/8	BLD	BLD	5/8	BLQ	BLQ
Heroin	4/8	3	BLQ–5	2/8	BLQ	BLQ
Ketamine	8/8	2	BLQ–4	0/8	BLD	BLD
Lysergic acid diethylamide (LSD)	4/8	BLQ	BLQ	5/8	BLQ	BLQ
MDA	0/8	BLD	BLD	2/8	BLQ	BLQ
MDEA	1/8	BLQ	BLQ	0/8	BLD	BLD
MDMA	8/8	19	BLQ–112	2/8	BLQ	BLQ
Methadone	8/8	2570	450–6071	4/8	109	BLQ–398
Morphine	8/8	24	15–36	5/8	BLQ	BLQ–1
Morphine-D-glucuronide	0/8	BLD	BLD	0/8	BLD	BLD
Nicotine	8/8	296 987	161 476–443 000	8/8	2380	575–5011
Norbenzoylecgonine	7/8	2	BLQ–4	8/8	2	BLQ–3
Norcocaine	8/8	BLQ	BLQ	5/8	BLQ	BLQ
Norcodeine	8/8	91	3–1356	7/8	11	1–418
Normorphine	7/8	2	BLQ–5	1/8	BLQ	BLQ
Paraxanthine	3/8	BLQ	BLQ	4/8	202	BLQ–701
Phencyclidine	0/8	BLD	BLD	0/8	BLD	BLD
THC	0/8	BLD	BLD	6/8	BLQ	BLQ
THC-COOH	0/8	BLD	BLD	8/8	BLQ	BLQ
THC-OH	0/8	BLD	BLD	0/8	BLD	BLD

BLD ($LOD_{influent} = 3$ ng/L, $LOD_{effluent} = 1$ ng/L); BLQ ($LOQ_{influent} = 6$ ng/L, $LOQ_{efflunet} = 3$ ng/L).
Occ., occurrence, number of samples in which the compounds were detected/number of samples examined.

based on previously reported validated analytical methods [6, 12]. For the occurrence of the licit drugs, the LC-MS/MS analysis was done on an Agilent HP 1100 HPLC (Palo Alto, CA, USA), equipped with an autosampler and connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column ($125 \times 2.0\,mm^2$, particle size $5\,\mu m$) preceded by a C_{18} guard column (4×4, $5\,\mu m^2$), both supplied by Merck. The illicit drugs were separated by ultra-performance LC tandem MS (UPLC-MS/MS) [12, 14]. It is noted that caffeine, cotinine, nicotine, and paraxanthine are included in the list of drugs investigated with the chromatographic method for analyzing the illicit compounds, and their concentrations are therefore presented along with those of the illicit drugs. The chromatographic separation was performed at 40°C on a $100 \times 2.1\,mm^2$ id, $1.7\,\mu m$ particle size Acquity BEH C_{18} column (Waters). The triple quadrupole mass spectrometer, Quatro Micro (Waters), was operated in electrospray positive ionization mode. The detailed mass spectrometer conditions, limit of detection (LOD) and method recovery for the entire list of all compounds were reported previously [6, 12].

3 Results and discussion

3.1 Occurrence of licit drugs in urban wastewater

Table 1 reports the mean and the range of the concentration of each licit drug in the three UWTPs. Out of 29 compounds analyzed with the particular chromatographic method, the presence of 22 in IWW was confirmed. The presence of a variety of licit drugs belonging to various chemical classes was confirmed in all samples. Antibiotics, β-blockers, analgesics, and NSAIDs were the major groups of compounds detected in the EWW. NSAIDs have shown the highest concentrations in the IWW, especially acetaminophen, ibuprofen, diclofenac, and mefenamic acid with mean concentrations of ca. 115, 2.8, 2.3, and 1.4 μg/L, respectively. This result is in fairly good agreement with previously reported studies for plants located in other countries [17].

As shown in Table 1, ibuprofen is the only compound determined at almost all sampling points at all three UWTPs. Its concentrations are in agreement with those reported in the literature [1, 18]. The presence of high concentrations of ibuprofen in UWTPs influent is not surprising, due to the amount of ibuprofen generally consumed with or without prescription [18]. In contrast, naproxen and ketoprofen were detected in only some of the samples and in lower concentrations than other compounds (Table 1).

The concentrations of clofibric acid, fluoxetine, mevastatin, paroxetine, loratadine, and lansoprazole were below the detection limits in all samples examined. As also previously reported by Fatta-Kassinos et al. [16], the concentrations of these compounds were found to be below the detection limit of the method in all samples examined. On the contrary, other studies performed for other treatment plants elsewhere have demonstrated that fluoxetine and clofibric acid are most often present even though at relatively low concentrations [8, 19, 20]. Mevastatin, paroxetine, loratadine, and lansoprazole are probably biodegraded or get adsorbed onto particles during the treatment [21], or even during transport in the sewage pipes, and it seems that identifying the potential biotransformation products of these drugs is very important in order to follow their environmental fate.

Carbamazepine was also present in almost all samples with higher concentrations in the treated wastewater than in the raw sewage at the UWTPs (Table 1 and Supporting Information Tables S2–S4) [1, 21]. The most possible explanation very often given on the increased concentration is the conversion of carbamazepine glucuronides and other conjugated metabolites to the parent compound by enzymatic processes during the treatment [7].

Antibiotics (i.e., erythromycin, azythromycin, sulfamethoxazole, and ofloxacin) and β-blockers (i.e., atenolol, sotalolol, metoprolol, and propranolol) were found in the EWW of all three UWTPs; a fact suggesting that they are bioresistant (Table 1). For some antibiotics such as sulfamethoxazole and trimethoprim, the concentration is reduced to a high extent during the purification process, especially after the secondary treatment. Ofloxacin is one of the substances with high concentrations at all three stages of the treatment process. The observed concentration level of ofloxacin is in accordance with previously reported values for other countries [19, 22].

Trimethoprim was detected in the EWW of UWTP II and III (Supporting Information Tables S3 and S4). The presence of trimethoprim is usually related to the detection of sulfamethoxazole since they are often consumed together as ingredients of the same medicinal products [9]. Sulfamethoxazole is another compound found in almost all samples examined. Overall, the mean concentration of sulfamethoxazole in the EWW (76 ng/L) (Table 3) falls within the range of below limit of quantification (BLQ) to 880 ng/L detected in Croatia, Europe, and 79–472 ng/L in Ohio, USA [1, 6]. It should be mentioned that sulfamethoxazole has been listed as one of the most often-used drugs in Cyprus and this presumably relates to its presence in almost all samples examined (Supporting Information Table S5).

3.2 Determination of mass loads of licit drugs

Table 3 presents the percentage removal efficiency and the influent mass loads of the target compounds. The mass loads were estimated by multiplying the IWW sample concentration by the flow rates, according to the equation

$$M = Q\ C \tag{1}$$

where Q is the daily flow rate of wastewater (L/day) and C is the concentration of the drugs in wastewater (ng/L). These values were then normalized by the population equivalent of each plant. The removal rates of each licit drug for each of the three UWTPs were estimated according to their concentrations in IWW, SWW, and EWW samples.

UWTPs I, II, and III were found to have comparable total inflow mass loads of licit drugs (sum of the loads of the 22 licit drugs, excluding nicotine, cotinine, caffeine, paraxanthine) (ca. ~2.5 g/day per 1000 inhabitants). The mass loads in the IWW samples for each drug (Table 3) were slightly lower or comparable to the results provided in a previous study [7]. As reported by Zorita et al. [8] the total mass load of drugs in the IWW samples in Sweden was 6.7 g/day per 1000 inhabitants (sum of the loads of 13 investigated drugs). The load for the 22 licit drugs determined in this study is slightly lower even if a higher number of compounds were studied in this work. As demonstrated in many studies, each UWTP exhibits a different behavior with regard to the removal efficiency of each drug. Hence, it is not possible to establish a trend referring to the removal efficiency of each UWTP as this is also the case of other studies [8].

Table 3. Average removal efficiency (%) and influent mass loads of the target licit drugs, grouped according to their therapeutic group (average value referring to all three UWTPs)

Therapeutic group	Compound	Average removal efficiency (%)		Mass load (mg/day per 1000 inhabitants)
		SWW	EWW	
NSAIDs	Acetaminophen	~100	~100	2117.2
	Diclofenac	22	66	47.0
	Ibuprofen	98	99	49.9
	Indomethacine	5	69	1.4
	Ketoprofen	82	93	8.4
	Mefenamic acid	65	95	26.5
	Naproxen	96	~100	0.7
	Propyphenazone	25	95	0.1
Lipid regulators and cholesterol lowering statin drugs	Bezafibrate	81	90	21.4
	Clofibric acid	n.d.	n.d.	n.d.
	Gemfibrozil	2	69	9.1
	Mevastatin	n.d.	n.d.	n.d.
	Pravastatin	61	96	1.2
Psychiatric drugs	Carbamazepine	−21	0	38.8
	Fluoxetine	n.d.	n.d.	n.d.
	Paroxetine	n.d.	n.d.	n.d.
Anti-ulcer agent	Lansoprazole	n.d.	n.d.	n.d.
Histamine H1 and H2 receptor antagonistics	Famotidine	53	99	23.8
	Loratadine	n.d.	n.d.	n.d.
	Ranitidine	52	95	3.8
Antibiotics	Azythromycin	n.d.	n.d.	n.d.
	Erythromycin	36	46	2.3
	Ofloxacin	−59	65	13.6
	Sulfamethoxazole	67	76	6.2
	Trimethoprim	75	97	1.3
β-Blockers	Atenolol	75	88	69.3
	Metoprolol	45	73	6.4
	Propranolol	−62	12	0.9
	Sotalolol	32	80	10.9

n.d., not detected.

Although the consumption of sulfamethoxazole is much higher than that of ofloxacin (Supporting Information Table S5), it is remarkable that the mass load of ofloxacin (13.6 mg/day per inhabitant) is higher compared to sulfamethoxazole (6.2 mg/day per inhabitant). Generally, unexplained variations of concentration over time were also observed for sulfonamide antibiotics, probably because of deconjugation processes that may occur during their contact with activated sludge [5].

3.3 Removal efficiency for licit drugs in UWTPs

As shown in Table 3, some compounds show negative values with regard to the removal rate. For example, propranolol, carbamazepine, and ofloxacin, present in almost all samples, showed higher concentrations in the SWW than in the IWW. In addition, individual values for diclofenac concentrations showed similar levels of concentrations in SWW and IWW samples (Supporting Information Tables S2–S4). This can be potentially attributed to the deconjucation of glucuronidated or sulfated diclofenac or due to desorption from particles [8, 23].

Generally, for antibiotics, low removal (<77%) was determined, except for trimethoprim (97%, Table 3). According to other studies, the high removal efficiency observed is mainly due to sorption to activated sludge [5, 8]. Concerning ofloxacin, its removal after the biological treatment was found to be negative (−59%). For fluoroquinolones, although being very hydrophilic and zwitterionic compounds, sorption to sludge/particles is most probably the main elimination process at the UWTPs [8]. As also previously reported, the observed removals of ofloxacin vary from 24 to 86% [8, 24].

A limited removal percentage was observed for indomethacine and gemfibrozil (<70%); these results are in agreement with those of other studies reporting that the removal of these drugs after biological treatment is usually incomplete [1]. In contrast, in the case of acetaminophen the removal efficiency was almost 100% after the treatment because acetaminophen undergoes biodegradation [18].

The β-blockers atenolol, sotalolol, and metoprolol were removed in UWTPs up to 70–90%, whereas poor removal efficiency was observed for propranolol. As previously reported in the literature, the removal of atenolol, metoprolol, propranolol, and sotalolol usually ranged from <10 to 46, <10 to 83, 0 to 96, and 15 to 36%, respectively [7, 25]. Their removal in UWTPs is most probably achieved through stereoselective biological degradation [7].

3.4 Occurrence of illicit drugs and their metabolites in urban wastewater

The illicit drugs and their metabolites that have been analyzed are those most commonly used worldwide, including amphetamines, opioids, and cocaine. A summary of the results (mean concentration of drugs and a range of concentration) in untreated and treated wastewater is shown in Table 2.

In the IWW samples, the presence of 22 illicit drugs (including caffeine, cotinine, nicotine, and paraxanthine) was confirmed.

Caffeine, paraxanthine (psychoactive drugs) and nicotine, cotinine (alkaloid drugs) are ones of the world's most widely consumed drugs, and they are considered legal and are unregulated in nearly all parts of the world. The alkaloids, caffeine, and nicotine show the highest concentration in all IWW samples in comparison with the other drugs. These results are in agreement with those reported in the literature [13]. Caffeine, methadone, nicotine and its main metabolite cotinine were determined in relatively high concentrations (BLQ to 5815 ng/L) in all EWW samples monitored in this study.

Morphine and codeine, which are also used as licit drugs to moderate pain and relieve cough, were detected in all IWW samples, up to concentrations of 24 ng/L and ca. 4.4 μg/L, respectively. In addition, in the present study their major metabolites normorphine and norcodeine were determined in all wastewater samples. As Boleda et al. [14] reported, codeine can probably be converted to norcodeine by N-demethylation. Morphine is partly excreted as glucuronide conjugates, but these conjugates are hydrolyzed back to morphine by β-glucuronidases of fecal bacteria in raw wastewater and during wastewater treatment [15]. It is important to highlight the fact that a part of the amount of morphine in the IWW can be attributed to the heroin consumption since morphine is a hydrolysis product of heroin, and not only to its direct use [11]. It has to be mentioned that the removal efficiency of these compounds seems to be varying, since the removal of codeine is almost 57%, while morphine was almost completely removed.

Cocaine and benzoylecgonine, the main metabolite of cocaine, were quantified in all IWW samples of UWTPs in concentrations between 3 and 37 ng/L (Table 2). These concentrations are lower than those detected in raw wastewater in other European countries [7, 13]. Among the amphetamines, 3,4-methylenedioxyethamphetamine (MDMA, ecstasy) was the most frequently detected and exhibited the highest concentrations in IWW samples (19 ng/L). On the contrary, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), compounds analogous with MDMA, were not determined at all.

An effort to estimate the initial load of the illicit drugs in the raw flows was made. Maximum loads in IWW were obtained for nicotine, cotinine, codeine, caffeine, methadone, and benzoylecgonine for each of the two UWTPs, with concentration levels between 583 and 1638, 1.9 and 6.7, 10.8 and 20.2, 24 and 118.1, 1.4 and 39.9, and 0.1 and 0.3 mg/day per 1000 inhabitants, respectively. The results of the analysis of the illicit drugs and the study of the literature show that their concentrations vary from one country to another, or from a wastewater treatment plant to another, as a function of drug consumption and also the sewage volume entering the UWTP.

4 Conclusions

The optimized analytical methods used in the present study, based on off-line SPE followed by LC-MS/MS, proved to be a concrete method for the determination and quantification of licit and illicit drugs in wastewater in UWTPs in Cyprus. Considerable differences are expected from the fact that drugs consumption varies significantly from location to location. From this study it is observed that the drug concentrations vary considerably due to the various processes and conditions applied at each treatment plant, depending also on the various physicochemical parameters of each compound. Hence, it is very difficult to establish a general trend for all compounds concerning their removal at UWTPs.

Generally, the present study demonstrated that both licit (i.e., antibiotics, β-blockers, analgesics, and NSAIDs, etc.) and illicit drugs (i.e., methadone, cotinine, etc.) were found in the EWW of all UWTPs; a fact suggesting that they are bioresistant. These compounds were determined in relatively high concentrations (licit drugs: BLD to 5520 ng/L and illicit drugs: BLD to 5815 ng/L) in all samples monitored in this study. For some drugs, their concentrations were reduced to a relatively high extent during the purification process, especially after the secondary treatment. However, they are still detected in the EWW. The licit drugs showing the highest concentrations in the effluents were carbamazepine (1820 ng/L), diclofenac (804 ng/L), and atenolol (460 ng/L). Concerning the group of illicit drugs, maximum concentrations in EWW were obtained for nicotine, codeine, caffeine, and paraxanthine, with mean concentration levels up to 2380, 1864, 1859, and 202 ng/L, respectively.

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