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ANALYSIS OF SOME ABUSED DRUGS AND PHARMACEUTICALS IN MUNICIPAL WASTE WATERS (EFFLUENTS)

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ABSTRACT

A study of myriads of chemical pollutants in aquatic environment was carried out from the effluent of Nottingham Sewage Treatment Works (STWs) in United Kingdom. Using a solid phase extraction - gas chromatography technique (SPE-GCMS), fifteen compounds detected in sampled STW effluents ranged between 1.1 - 33.5 ng L^{-1} with percentage recoveries of 78.6 – 97.8%. The most abundant compounds found in the final effluent were nicotine, ibuprofen, codeine, ephedrine, procaine, benzoylecgonine, lidocaine, and caffeine, with mean concentrations of 21.4 ± 6.4, 16.7± 4.8, 15.1 ± 6.3, 10.1 ± 3.9, 9.1 ± 3.4, 5.6 ± 3.1, 5.3 ± 5.2 and 5.2 ± 1.9 ng L^{-1}, respectively. The instrumental limits of detection (LODs) from 0.1 – 1.7 ng L^{-1}, with standard deviations (STDs) of 1.1– 21.4% for all the compounds were also observed. The levels of illicit drugs and abused pharmaceuticals detected from the effluents showed the occurrence of these drugs and the Nottingham STW as one of the main transport routes to the receiving environment. These findings have further shown that chemicals in effluent get to the environment due to their incomplete removal from the STW. Persistence influx of these pollutants into the aquatic environment may have implication on ecosystems.

Keywords: *effluent, environment, pharmaceutical, pollutant, wastewater.*

INTRODUCTION

In recent years, there have been global concerns about the emerging levels of illicit drugs apart from already abused pharmaceuticals in the aquatic environment. The escalating levels of these drugs have prompted the need for proper monitoring of their prevalence in order to stem its social and environmental impacts (Beyer *et al.,* 2009; Kasprzyk-Horden *et al.,* 2009; Kasprzyk-Horden *et al.,* 2010; Patrolecco *et al.,* 2015). Environmental monitoring and risk assessments have shown the sewage treatment works (STWs) as one of the potential routes through which the levels of consumption by a local population can be estimated. Several studies have reported the plethora of illicit drugs from their common classes such as cocaine, opioid, cannabinoid, amphetamine, lysergic diethylamide (LSD) and hallucinogen (Karolak *et al.,*2010., Metcalfe *et al.,* 2010; Postigo *et al.,* 2010).

The biodegradability and non – biodegradability or resistance to degradation have increased the environmental risk factors of the ecosystem due to the hydrophobicity/lipophobicity properties of these drugs. The myriads of existing pharmaceuticals coupled with the menace of use and abuse of illicit drugs have necessitated the establishment of many international agencies saddled with the responsibilities to monitor the production, transport, unlawful possession and usage of the 'controlled' substances. The substances considered risky for human health and social well-being of the society have different legislation in many countries with the hope to curb the menace but the concerted efforts have not fully achieved for their purpose. In spite, the nefarious activities of the consumers are on the increase and the hidden natures of the business have helped its purported widespread and escalating consumption (Mustapha, 2013; Postigo et al., 2011; Ra *et al.,* 2011).

Until recently the idea of Daughton (Daughton and Ternes. 1999), using intrusive approach provided information on the community consumption of illicit drugs of whose idea was later demonstrated by Zuccato (Zuccato *et al.,* 2008a), nearly nothing was in the public domain about the estimation and levels of illicit drugs in the environment. Today, the illicit drugs are similarly surviving like other pharmaceuticals and medicinal drugs have been reported by several studies but the extent and spread differs from one location to another (Zuccato *et al.,* 2000), Therefore, it is the aim of the present study to assess the levels of these illicit drugs and abused pharmaceuticals in the effluents to receiving waters by making exposure data to potential threats it constitutes to the environment as well to the appropriate authorities involved in fighting and controlling drug menace.

MATERIALS AND METHODS

Standard compounds of ibuprofen, caffeine, lidocaine, cocaine, codeine, amphetamine, ecgonine methylester, benzoylecgonine, ephedrine, methadone, nicotine, 6-acetylmorphine, diacetylmorphine, diazepam and procaine (Sigma Aldrich, Gillingham Dorset, UK) and LGC standards (Teddington Middlesex, UK). Analar grade hydrochloric acid (HCl), ammonium hydroxide (NH4OH) and methanol (MeOH), N, O, bis (trimethylsilyl) trifluoroacetamide (BSTFA with 1% trimethylchlorosilane, TMCS (Cerrilliant, Round Rock, TX, USA). HLB® sorbent in a 47mm SPE disc format and disc holder (Waters, Elstree Herts, UK) and a Phenomenex SPE Vacuum Manifold with 12 ports (Macclesfield Cheshire, UK).

The Nottingham STW is located at Stoke Bardolph in the East Midlands; it treats the sewage of about 170 million litres per day using activated sludge. It serves over 500 000 people. A total of 16 hours is used for wastewater treatment stages from influent wastewater to effluent including recycling before discharging it to River Trent. The STW further removes 330 tonnes of plastics and paper as well as 2000 tonnes of grit per year (Mustapha, 2013).

The appropriate sites for sampling operations along Stoke Bardolph STW in Nottingham were identified and collected samples were analysed to assess the levels of compounds from the discharges. Treated wastewater samples were collected biweekly outside heavy rain period to avoid dilution and possible overflow that can result into analyte losses. The grab waste water samples was stored at 4oC (pH = 2 with 37% HCl) in Winchester glass bottles to prevent degradation by bacterial activity during storage. Systemic sampling approach was adopted for all the eight sampling expeditions of wastewater at every two weeks interval for four months to minimise bias.

Sample analysis

100 ug L^{-1} of mixed compounds (ibuprofen, caffeine, lidocaine, cocaine, codeine, amphetamine, ecgonine methylester, benzoylecgonine, ephedrine, methadone, nicotine, 6-acetylmorphine, diacetylmorphine, diazepam and procaine) were used to spike 1 litre of wastewater for a recovery experiment and analysed in gas chromatography mass spectrometry (GCMS). However, the data obtained from the recovery values were not re-computed.

The solid particles were removed by glass filter

(Whatman GF-C; 1.2 µm), adjusted to appropriate pH of 6 using NH_4OH and HCl before employing Oasis HLB (500mg/6 mL) solid phase extraction. After soaking the cartridge and pre – conditioning with methanol, the eluates obtained from 1 litre of wastewater were concentrated, derivatized to their respective trimethylsilyl derivatives in three steps and put in 250 mL glass vials for GCMS analysis The appropriate dilutions of 100 ug mL-1 of mixed compounds to standard concentration range of 2-10 ugL-1 were obtained for linear calibration curves using five point curves with ($0.9534<r^2<0.9998$) higher than 0.99. The LODs of various drugs were separately calculated (Mustapha, 2013). Gas chromatography mass spectrometry operational settings: Gas chromatograph: Agilent 6890 GC; Mass selector detector: Agilent 5975 inert XL (MSD: 2564.7 eV); Capillary column: HP5-MS (30.0 x 0.25mm x 0.25 um film thickness); Carrier gas: Helium (1 mL min^{-1}); Sample injection: Splitless mode; Temperature: 50°C (hold 2 min), rise to 300oC at 10oC min-1, held at 300°C for 3 min; Quantitation area: Total ion correspond (TIC)

MS ionization mode: Electron impact (EI) at 70eV; EI mass spectra scan mode: Single ion mode (scan range 45- 550m/z); MS run time: 30 min and Standard software: Agilent Chemstation (Manufacturer). The retention times of major mass fragments (m/z) of compounds for characteristic identification as well as for both quantitative and qualitative determinations are shown in Table 1.

RESULTS AND DISCUSSION

Table 1: Recovery data in spiked water.

Analytes	Two levels of concentration		Mean	Retention Time
	2.0 (ng/L)	4.0 (ng/L)		
6- acetylmorphine	74.5	82.7	78.6	24.6
Amphetamine	91.6	98.4	95.0	10.0
Benzoylecgonine	92.5	92.2	92.4	17.4
Caffeine	88.5	79.2	83.9	18.2
Cocaine	92.6	90.2	91.4	21.9
Codeine	89.6	85.3	82.4	24.9
Diacetylmorphine	81.0	83.7	82.4	26.4
Diazepam	76.6	86.0	81.3	24.0
Ecgonine methyl ester	85.2	90.8	88.0	11.7
Ephedrine	78.4	81.9	80.2	14.6
Ibuprofen	88.8	86.7	87.8	15.6
Lidocaine	86.0	91.0	88.5	18.7
Methadone	97.5	87.6	97.8	21.4
Nicotine	83.6	91.7	87.7	20.8
Procaine	83.9	80.6	87.5	20.3

Figure 1 show the solid phase extraction - gas chromatography mass spectrometry (SPE-GCMS) performance with respect to the retention times of all the 15 compounds for identification and quantification. In Table 1, recovery percentages at two levels of concentration of compounds (2.0 and 4.0ng/L) at triplicate runs show the mean average recoveries of generally above 70% (78.6 – 97.8%). Several studies in the literature have shown many recoveries of some illicit compounds such as cannabinoids, opiates (85 – 90%) and cocaine (69 – 105%) and these values compares well with the present work (Baker *et al.,* 2011; Gheorge *et al.,* 2008; Harman *et al.,* 2012., UNODC, 2017)

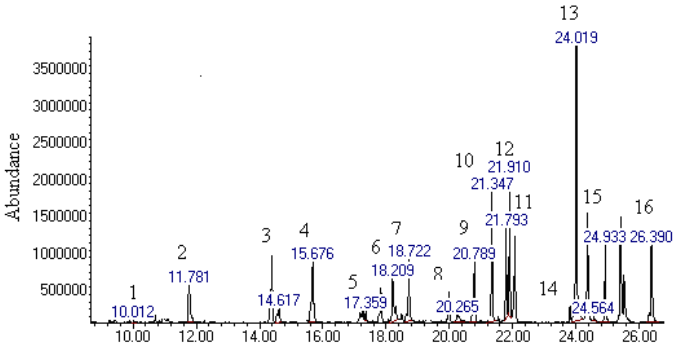

Figure 1: Total ions chromatogram of standard analytes

(1 = Amphetamine-N-TMS; 2 = Ecgonine methyl ester-O-TMS; 3 = Ephedrine; 4 = Ibuprofen-O-TMS; 5 = Benzoylecgonine; 6 = Caffeine-N-TMS; 7 = Lidocaine; Caffeine-N-TMS; 8 = Procaine-N-TMS; 9 = Nicotine; 10 = Methadone; 11¬ = Cocaine; 12 = Diazepam; 13 = 6-acetylmorphine-O-TMS; 14 = Codein -N-TMS 15 = Diacetylmorphine).

In similar developments, the psychoactive drugs recoveries in wastewaters between 70 - 101% from determination of cannabis, amphetamines, cocaine and their metabolites were reported in wastewaters (Hedgespeth *et al.,* 2012). Recoveries of 70 -120% after SPE enrichment have also been reported with precision of ≤ 20% for methadone, cotinine, caffeine, nicotine, ketamine and paraxanthine (Mustapha, 2013)

Compounds detected in Nottingham STW effluent.

Table 2 show the 15 compounds that were detected from biweekly samplings from Nottingham STW effluents including ibuprofen, caffeine, lidocaine, cocaine, codeine, amphetamine, ecgonine methylester, benzoylecgonine, ephedrine, methadone, nicotine, 6-acetylmorphine, diacetylmorphine, diazepam and procaine with their corresponding concentrations as presented. The recomputed values of the analytes with various percentage recoveries were not included in order to present the raw concentrations in the effluent.

The occurrence of drugs in wastewaters with the mass spectrometer in electron impact (EI) recorded in scan mode (scan range 45-550 m/z) gave abundant molecular ion of each compound and two precursor ions: ibuprofen (278>160; >278>73), caffeine (194>109; 194>67), lidocaine (234>86; 234>58), cocaine (303>182; 303>82), codeine (299>178; 371>73),amphetamine(206>116;206>73),),ecgonine methyl ester (199>96; 199>82), benzoylecgonine (290>168; 290>150), ephedrine (230>179; 230>58), methadone (283>197; 283>180), nicotine (161>84; 161>131), 6-acetylmorphine (341> >282; 341>229), diacetylmorphine (369>310; >369>268), diazepam (285>256; 283>221) and procaine (235>99; >235>88). The characteristic fragmented ions (m/z signals) were used for identification of analyte. Table 2 show cocaine (1.9 ng L^{-1}) with LOD of 0.8 ng L^{-1} but fell within the range of 0.9 -10.7 ng L^{-1} that were reported (Postigo et al., 2010; Karolak et al., 2011). Also, studies have reported 1-100 ng L^{-1} and 47 ng L^{-1} of cocaine in effluents (Daughton and Ternes, 1999; Zuccato., 2008a). Similarly, cocaine concentrations of 10 ng L^{-1} in surface water and 1.2-26.0 ng L^{-1} were obtained from three rivers. In Dublin, Ireland cocaine in the range of 25 – 248 ng L^{-1} in 70% of the river waters collected were detected.

Benzoylecgonine, a principal metabolite of cocaine (5.6 ± 3.1ng L^{-1}) compared with 25 ± 5 ng L^{-1} with ≥ 90% recoveries obtained from River Po in Italy but the result of 77 ng L^{-1} was much greater than the values from Germany STP effluents. In Dublin, a river water had (22 – 290 ng L^{-1}) of benzoylecgonine. The strict legislation in United Kingdom against controlled drugs could be responsible for low detection of drugs. Another metabolite of cocaine wass ecgonine methyester (1.1 ± 1.4 ng L^{-1}) had LOD of 0.3 ng L^{-1} but the occurrence of amphetamine, cocaine, ecgonine methylester and benzoylecgonine were expectedly found in some of the weekend sampling periods being recreational drugs (Metcalfe *et al.,* 2010; Postigo *et al.,* 2010; Karolak *et al.*,2010). Opiates in some urban waters included morphine (80-200 ng L-1) and 6-acetylmorphine (10 ng L^{-1}), whereas from current study ephedrine, 6-acetylmorphine, diacetylmorphine and codeine mean concentrations of 10.1 ± 3.9 ng L^{-1}, 3.4 ± 2.8 ng L^{-1}, 2.4 ± ng L^{-1} and 15.1 ± 6.3 ng L^{-1} with their corresponding LODs (Table 2), respectively. All the analytes concentration ranges found in Nothingham STW was much lower compared to the values earlier reported in the literature (Metcalfe *et al.,* 2010; Postigo *et al.,* 2010; Karolak *et al.,*2010). Amphetamines that have been found in surface waters in other places in the literatures were (110 - 210 ng L^{-1}) and (20 ngL^{-1}), but the amphetamine mean levels of 4.8 ± 1.5 ng L^{-1} from our study falls within the values of (0.4 - 2100 ng L^{-1}) found in municipal wastewaters from North Eastern Spain. Procaine is used as anaesthetic its mean concentration in wastewater was 9.1 ± 3.4 ng L^{-1} and had LOD of 1.2 ng L^{-1} its presence in the effluent of Nottingham STW was significantly different. Diazepam being abused pharmaceutical had concentrations between 3.9 – 9.9 ng L^{-1} and compares with 38 ng L^{-1} - 127 ng L^{-1} of diazepam found in Dublin, Ireland. Tempazepam concentration of 1 – 10 g L^{-1} was other substance reported using LC-MS-MS in the wastewater (Mustapha, 2013)

Table 2: Concentration of compounds (ng/L) from Nottingham STW effluents (n = 3)

Biweekly Sampling Periods of Nottingham Effluents for 4 Months											**Major ions for substance identification (m/z)**		
Compound	**1ST SMP**	**2ND SMP**	**3RD SMP**	**4TH SMP**	**5TH SMP**	**6TH SMP**	**7TH SMP**	**8TH SMP**	**Mean ± STD**	**LOD (ng/L)**	**Molecular ions**	**Precursor ions**	**Product ions**
6- acetylmorphine	1.6	2.3	4.0	9.1	4.3	1.1	1.3	< LOD	3.4± 2.8	0.9	341	282	229
Amphetamine	4.3	5.1	5.3	7.3	2.8	3.3	3.9	6.2	4.8± 1.5	0.3	206	116	73
Benzoylecgonine	5.4	5.4	5.2	3.9	2.7	12.2	4.6	< LOD	5.6 ± 3.1	1.6	290	168	150
Caffeine	8.6	5.4	7.2	4.3	4.6	5.6	3.8	2.4	5.2± 1.9	0.6	194	109	67
Cocaine	1.9	1.5	1.2	1.6	1.6	< LOD	< LOD	< LOD	1.6 ± 0.3	0.8	303	182	82
Codeine	11.2	12.2	12.0	15.8	12.6	14.4	12.1	30.2	15.1± 6.3	0.4	299	178	73
Diacetylmorphine	< LOD	< LOD	3.8	2.9	1.2	< LOD	< LOD	1.5	2.4± 1.2	0.9	369	310	268
Diazepam	3.9	9.9	1.3	4.7	5.9	5.2	1.5	2.9	4.4± 2.8	1.3	285	256	221
Ecgonine ME	0.7	< LOD	1.9	1.6	0.9	0.9	< LOD	0.3	1.1± 1.4	0.3	199	99	82
Ephedrine	4.1	15.3	15.8	8.9	7.7	10.1	11.1	8.1	10.1± 3.9	0.1	230	179	58
Ibuprofen	15.9	14.9	8.9	18.6	16.9	15.0	19.2	25.9	16.7± 4.8	0.9	278	160	73
Lidocaine	17.4	6.4	1.5	2.7	2.2	5.8	2.5	3.5	5.3± 5.2	1.5	234	86	58
Methadone	4.2	3.6	4.6	2.5	4.6	1.4	< LOD	< LOD	3.5± 1.3	1.3	283	197	180
Nicotine	22.7	18.7	18.9	12.7	16.1	33.5	22.7	25.5	21.4± 6.4	1.7	162	133	84
Procaine	16.9	7.2	9.3	7.9	8.2	5.2	9.2	9.2	9.1± 3.4	1.2	235	99	88

Note: SMP = sampling period; n = triplicate determinations; STD = standard deviation; STW = sewage treatment work; LOD = limit of detection.

Nicotine is from tobacco and their higher presences in influents suggest possibility of its wider consumption. The present wastewater sampling indicated nicotine (21.4 ± 6.4 ng L^{-1}), caffeine (5.2 ± 1.9 ng L^{-1}) and ibuprofen (16.7 ± 4.8 ng L^{-1}) with LODs of 1.7, 0.6 and 0.9 ng L-1, respectively. The nicotine values were higher than the (2.6 - 5.7 ng L^{-1}) reported in Spain STW but lower than 175 – 198 ng L^{-1} detected in rivers (Han *et al.,* 2017; Hedgespeth *et al.,* 2012; Kaspryk-Horden *et al.,* 2012; Patrolecc *et al.,* 2015; Ra *et al.,* 2011)

Systematic samplings in every two weeks ensured the representative samples were collected but the possibility of dilution of effluents at the discharge point by the receiving waters may cause concentration gradient with distance. The sampling strategy may introduce uncertainties in the actual levels of analytes due to the possibilities of some substances that may escape detection, but the presence of the analytes in varying amounts further confirmed the challenges of sewage works as major routes through which pollutants discharges enters the environment. Unlike pharmaceuticals, illicit compounds are new challenges of chemicals and paucity of experimental information has made their removal and treatability in STWs difficult.

However, appropriate sampling strategy such as the one employed at the determined intervals of two weeks was conducted at Stoke Bardolph STW Nottingham effluents to allow the evaluation of the new trends of analytes and the results might improve treatment capability of STWs.

CONCLUSION

This study confirms necessary capabilities of analytical methodologies for the determination of drugs in wastewaters using SPE-GCMS. The presence of 15 different compounds: ibuprofen, caffeine, lidocaine, cocaine, codeine, amphetamine, ecgonine methylester, benzoylecgonine, ephedrine, methadone, nicotine, 6-acetylmorphine, diacetylmorphine, diazepam and procaine were found. The compounds detected in effluents ranged between 0.3 and 33.5 ng L^{-1} with percentage recoveries from 78.6 – 97.8%, using SPE - GCMS. The instrumental limits of detection (LODs) ranged from 0.1 – 1.7 ng L^{-1}, and standard deviation (STD) values of 1.1– 21.4%.

The most abundant compounds found in the final effluents were nicotine, ibuprofen, codeine, ephedrine, procaine, benzoylecgonine, lidocaine, and caffeine, with mean concentrations of 21.4 ± 6.4, 16.7± 4.8, 15.1 ± 6.3, 10.1 ± 3.9, 9.1 ± 3.4, 5.6 ± 3.1, 5.3 ± 5.2 and 5.2 ± 1.9 ng L^{-1}, respectively.

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