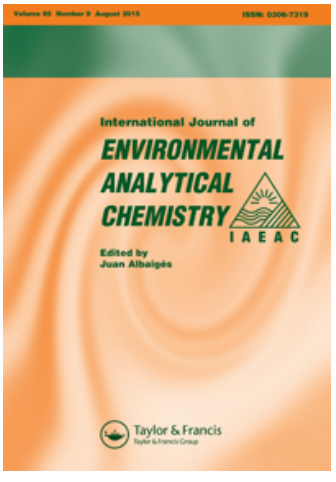

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Simultaneous extraction and determination of pharmaceuticals and personal care products (PPCPs) in river water and sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry

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Simultaneous extraction and determination of pharmaceuticals and personal care products (PPCPs) in river water and sewage by solid-phase extraction and liquid chromatography-tandem mass spectrometry

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This study features the simultaneous extraction and quantification of 18 pharmaceuticals and personal care products (PPCPs). This is a pioneering method for the quantification of acetaminophen, sulfamethoxazole, diclofenac, atenolol, metoprolol, diethyltoluamide and oxybenzone in atmospheric pressure chemical ionisation mode. The method was validated for high repeatability and reproducibility with relative standard deviations less than 10%. Instrument quantification limits for PPCPs were within the range of 0.05–1.0 μg L^{-1}, and the method quantification limits (MQLs) for ultrapure water were within the range of 0.3–15 ng L^{-1}. All samples were extracted using Oasis© hydrophilic–lipophilic balanced cartridges with optimised sample size and extraction conditions. Good accuracy was demonstrated, with solid-phase extraction recoveries above 80% for most PPCPs. In environmental matrices, the MQLs for river water, sewage treatment plant (STP) effluent and STP influent were 4–25, 10–153 and 38–386.5, respectively. The method was successfully applied to investigate occurrences of persistent PPCPs in Malaysian river and sewage samples.

Keywords: Pharmaceuticals; personal care products (PCPs); environmental pollution; solid-phase extraction (SPE); liquid chromatography-tandem mass spectrometry (LC-MS/MS); atmospheric pressure chemical ionisation (APCI)

1. Introduction

Pharmaceuticals and personal care products (PPCPs) have been an emerging class of pollutants in the past decade due to their ubiquitous nature, toxicity and persistence in the environment. The term 'emerging pollutants' describes the entrance or generation of pollutants into the environment in appreciable amounts, having a significant degree of persistency and exhibiting detrimental effects on organisms [1].

The occurrence of PPCPs in terrestrial and aquatic environments has exposed non-target organisms to PPCPs. The ecotoxicity of reported PPCPs includes the development of antibacterial resistance in microorganisms such as *Staphylococcus aureus*; growth inhibition and retardation in phytoplankton when exposed to antibacterial compounds; and smaller adults, reduced egg production and abnormal growth in copepods [2]. Several PPCPs have been classified as potential endocrine disrupting compounds, capable of causing sexual

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underdevelopment, infertility, altered or reduced sexual behaviours, attention deficiencies or hyperactivity, altered thyroid or adrenal cortical function, increased incidences of certain cancers and birth defects [3].

A national reconnaissance was created when the occurrence of PPCPs was investigated in 139 streams across 30 states in the USA [4]. PPCPs were detected in 80% of the streams sampled. Scientific interest in PPCPs has escalated in the past decade, with a significant increase in the number of studies conducted each year. These studies are focused on the distribution, degradation and ecotoxicity of PPCPs to aquatic organisms, as well as their end-point in potable drinking water. Relatively fewer studies on soils, sludge and sediments have been reported [3].

Analysis of PPCPs in environmental waters typically involves extraction and pre-concentration of sample prior to instrumental quantification. Previously, sample extraction was carried out using liquid-liquid extraction. Owing to its tedious and labour intensiveness, many now opt for solid-phase extraction (SPE). SPE allows simultaneous extraction, sample clean up and sample concentration. Literature had shown Water Oasis© hydrophilic–lipophilic balanced (HLB) to be the SPE sorbent of choice particularly for extraction of multi-residue PPCPs. Its excellent performances were reported for extraction of polar and non-polar compounds as well as acidic, basic and neutral compounds [5–8]. Moreover, it had also been successfully employed to extract complex matrices [9,10].

Several analytical approaches have been developed for quantification of various PPCPs. These include the use of immunoassay [11], gas chromatography-mass spectrometry [12], gas chromatography-tandem mass spectrometry [13], liquid chromatography-mass spectrometry [14], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [15] and online SPE LC-MS/MS [16–18]. Liquid chromatography (LC) is more suitable for compounds with low volatility such as PPCPs. Quantification for PPCPs using GC requires additional derivatisation procedure to decrease analytes' polarity, enhance volatility and increase thermal stability. Its drawbacks are lower analyte recovery and hazardous exposure to toxic derivatisation reagents [19]. Moreover, LC-MS/MS demonstrates enhanced selectivity in complex matrices and better sensitivity [20]. Thus, LC-MS/MS is the instrumental quantification of choice for trace PPCPs. Despite significant number of methods, there is no uniform list of PPCPs as pharmaceutical consumption as well as usage pattern of personal care products (PCPs) differs from country to country.

Previous studies have reported the environmental occurrence of the top 20 and several top over the counter pharmaceuticals utilised in Malaysia in the year 2010 [21–23]. Evidently, out of 23 quantified pharmaceuticals, only a handful persisted in the environment, these being atenolol, acetaminophen, metoprolol, diclofenac, levonorgestrel and norethindrone. A separate study conducted by Al-Qaim et al. [9] reported high occurrences of 3 PPCPs (caffeine, diclofenac and levonorgestrel) out of 11 PPCPs which were analysed. These persistent PPCPs were pooled and adopted into our method development.

Recently, Malaysian researchers are concerned on the heavy use of insecticide containing diethyltoluamide (DEET) to combat increasing fatality of dengue fever [24]. Other PPCPs of concern include oxybenzone owing to its high solar ultraviolet radiation as well as natural hormones from human and veterinary sources. A new list of PPCPs of interest had been compiled based on their pattern of consumption in Malaysia as well as literature reported environmental occurrences and persistency [1,25,26]. The list includes eight pharmaceuticals, three PCPs, four natural hormones and three synthetic hormones. It is worthwhile to note that four hormones in this study are listed under United States Environmental Protection Agency (USEPA) Contaminant Candidate List 3, namely, 17β-estradiol, estriol, estrone and 17α-ethynylestradiol [27]. Detailed information of the analyte's chemical abstract service, molecular weight, octanol-water partition coefficient and pKa are available in Table S1 (supplemental data).

Thus, this study aims to develop a new, selective and sensitive method for the simultaneous extraction and quantification of PPCPs using LC-MS/MS in atmospheric pressure ionisation mode, which is less susceptible to matrix effects (ME) compared to electrospray ionisation (ESI) [28–31]. The developed method was applied for the quantification of PPCPs in environmental waters such as river water, sewage treatment plant (STP) influents and effluents using atmospheric pressure chemical ionisation (APCI) mode.

2. Experimental

2.1. *Chemicals and materials*

The reference standards estradiol (17β-estradiol), estriol, estrone, 17α-ethynylestradiol, diclofenac sodium salt, atenolol, (±)-metoprolol (+)-tartrate salt, gemfibrozil, acetaminophen, naproxen, oxybenzone (2-hydroxy-4-methoxy benzophenone), D(-)-norgestrel (levonorgestrel), 19-norethindrone, sulfamethoxazole, progesterone, and trimethoprim above 97% purity were purchased from Sigma-Aldrich (Germany). DEET (99%) was purchased from Dr Ehrenstorfer GmbH (Germany), and caffeine (99.9%) was purchased from Alfa Aesar (USA). Isotope-labelled standards ($^{13}C_3$-caffeine, $^{13}C_2$-17α-ethynylestradiol, $^{13}C_3$-trimethoprim, $^{13}C_3$-sulfamethazine) used as surrogates and internal standards (SIS) were purchased from Cambridge Isotopes Laboratories (USA). Diclofenac-d_4 was purchased from Toronto Research Chemicals Inc. (Canada). Individual stock standard solutions (1000 mg L^{-1}) were prepared monthly. Working standards were prepared weekly by diluting stock standards to a final concentration of 10 mg L^{-1}. All standard solutions were prepared in 100% high performance liquid chromatography (HPLC) grade methanol. Stock and working standards were kept sealed in ampoules and are refrigerated in upright chest freezer at −25°C. HPLC grade methanol, formic acid, methyl-tert-butyl-ether (MTBE), sulphuric acid and sodium hydroxide were purchased from Thermo Fisher Scientific Inc. (USA). Ascorbic acid (>99%) and sodium azide (≥99%) were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade dichlorodimethylsilane (DMDCS) (≥99%) was purchased from Acros Organics (Belgium). HPLC grade acetonitrile (ACN), as well as analytical grade methanol, acetone and hexane were purchased from Merck KGaA (Germany). Ultrapure water was produced using a Millipore Integral System.

All samples were filtered prior to SPE extraction using Whatman glass filter GF/F 0.7 µm, 47 mm from Whatman International Ltd (Springfield Mill, UK). Millipore nylon membrane filters 0.2 µm, 47 mm was used to filter all organic solvents prior to LC-MS/MS quantification and was purchased from Millipore (Massachusetts, USA). In addition, non-sterile membrane syringe filters 0.22 µm, 4 mm was used to filter reconstituted samples after SPE extractions, and was purchased from Membrane Solutions LLC (Texas, USA). Oasis© HLB 3 cc/60 mg cartridges were purchased from Waters (MA, USA). SPE extraction was carried out using an ISOLUTE VacMaster SPE vacuum manifold (UK). Nitrogen gas (N_2) (99.9%) was used to dry samples and all glassware after silanisation. N_2 was purchased from Linde Malaysia Sdn. Bhd. (Malaysia). All glassware was washed and prepared in accordance with EPA Method 1694 [32]. All glassware was also silanised prior to usage.

2.2. *Samples*

Ultrapure water was used as a blank reference sample. For the purpose of method development and method validation, river water was collected upstream of the Langat River Basin (N03° 12′ 53.9′, E101° 53′ 1.06′E), where minimal impact from anthropogenic activities was expected. River water samples were collected in 1 L white non-transparent plastic bottles. Sewage samples

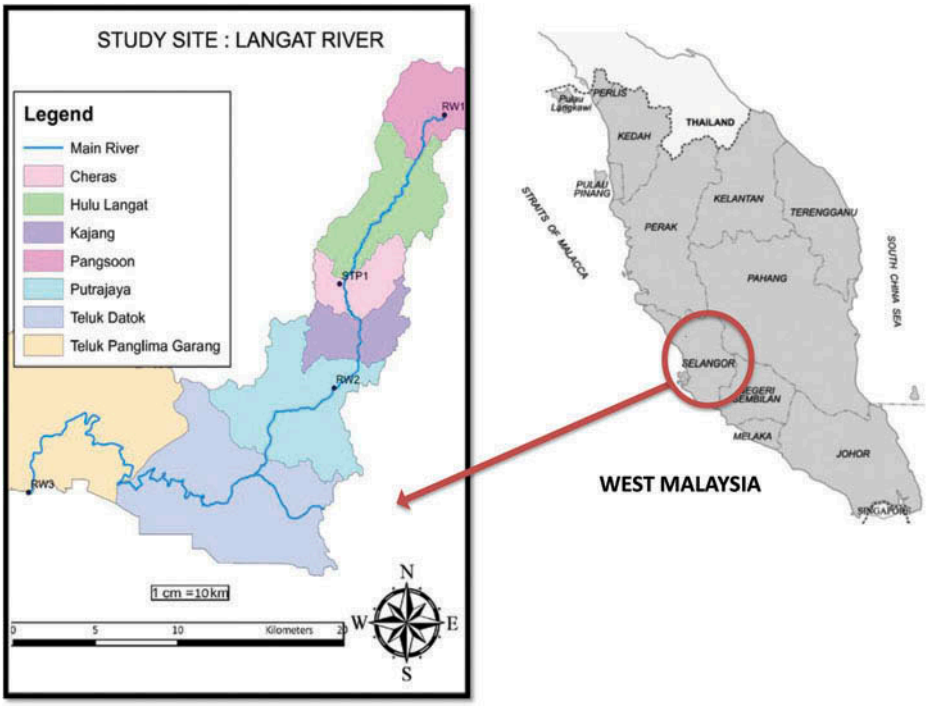

Figure 1. Map of river water and STP sampling locations in the Langat River Basin.

were collected using the ISCO 3700 Portable sampler as 48-hour time-paced multiple bottle composite. Samples were collected every 4 hours. Thus, each 1 L composite sample consisted of 12 smaller samples were collected in Extended Aeration STP.

All samples were transported on ice at approximately 4°C. Upon reaching the laboratory, samples were acidified to pH 2 using 37% sulphuric acid and preserved with 1 g L^{-1} sodium azide to minimise microbial degradation as well as 50 mg/L ascorbic acid to quench any residual oxidant. Samples were filtered using Whatman GF/F filter paper to remove any suspended particulate matter. Typically, samples were extracted within 24 hours.

The developed method was applied to detect PPCPs in the Langat River Basin; three river water locations (RW1 upstream, RW2 midstream and RW3 downstream) and one sewage treatment plant (STP1) were sampled. A map of sampling locations is shown in Figure 1.

2.3. *Procedures*

2.3.1. *Solid-phase extraction*

Samples of 150 mL of river water, 150 mL of STP effluent, 100 mL of STP intermediate and 50 mL of STP influent were spiked with SIS mixture of 200 ng mL^{-1} of $^{13}C_2$-17α-ethynylestradiol, diclofenac-d_4, $^{13}C_3$-trimethoprim, $^{13}C_3$-sulfamethazine and $^{13}C_3$-caffeine.

The Oasis HLB 3 cc/60 mg cartridges were conditioned with 3 mL of MTBE, 3 mL of methanol and 3 mL of acidified ultrapure water (acidified to pH 2 by formic acid). Each conditioning procedure was carried out by gravity elution followed by drying using a vacuum manifold. Samples were loaded into cartridges at rate of 10 mL min^{-1}. SPE cartridges were then

washed with 3 mL of ultrapure water, which had been acidified by formic acid to pH 2 and then vacuum-dried for 15–20 min.

Subsequently, PPCPs were eluted into 15 mL centrifuge tubes using 3 mL of a methanol: MTBE mixture (10:90), followed by 3 mL of methanol. To achieve optimum results, elution was carried out by gravity flow. The extract was then dried under a stream of nitrogen gas until near dryness. The extract was reconstituted with 250 μL of a mixture consisting of ultrapure water with 0.1% formic acid: methanol (75:25). The final extract was filtered using a 0.22 μm, 4 mm-diameter nylon syringe filter and transferred into a 2 mL amber glass vial fitted with a 250 μL silanised vial insert.

2.3.2. *Liquid chromatography-tandem mass spectrometry*

Accela high-speed LC interface to TSQ Quantum Ultra triple stage quadrupole (QqQ) mass spectrometer (Thermo Scientific, CA, USA) was used to quantify the PPCPs. Thermo Scientific Hypersil GOLD whose dimension is 50 mm × 2.1 mm, 1.9 μm, was used in this study. The mobile phase consisted of a mixture of three solvents: 0.1% formic acid in ultrapure water (A), methanol (B) and ACN (C). A flow rate of 100 μL min^{-1} was used. The gradient was as follows: 90% A, 9% B and 1% C held for 1 min and then increased linearly to 1% A, 79% B and 20% C by the 15th min and held for 4.5 min; the gradient was then decreased linearly to its initial concentrations and held constant for 5 min with the aim of ensuring equilibration. The LC-MS/MS dwell volume was 65 μL. Quantifications in both APCI positive and negative were carried using LC-MS/MS fast switching mode of less than 25 ms. The total run time was 25 min. The sample injection volume was 10 μL. Other optimised conditions include the discharge current (4.8 μA), vaporiser temperature (250°C), capillary temperature (250°C), sheath gas pressure (20 arb units) and auxiliary gas pressure (5 arb units). The incorporation of the final source parameters, compound parameters, mobile phase, gradient elution and reconstitution solvents for the quantification of PPCPs in HPLC methanol yields chromatograms as shown in Figure 2. Optimised APCI and MS/MS parameters were adopted for selected reaction monitoring (SRM) in LC-MS/MS analysis.

2.3.3. *Quantification and method validation*

Validation of instrumental intra-day precision was carried out by quantifying a mixture of 100 μg L^{-1} of PPCPs in methanol at three intervals of morning, noon and evening on three consecutive days using LC-MS/MS. Instrumental inter-day precision was verified by quantifying a mixture of 100 μg L^{-1} of PPCPs in methanol on five consecutive days using LC-MS/MS. Precision of the method was calculated by measuring the dispersion of sets of data under repeatability or reproducibility conditions according to the following equation:

$$\text{Precision} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100\%. \quad (1)$$

Instrument detection limits (IDLs) and instrument quantification limits (IQLs) were validated by direct injection of decreasing concentrations of the PPCPs. The IDL and IQL of each target compound were determined at signal-to-noise (S/N) ratios of 3 and 10, respectively. The instrument was calibrated using a seven-point calibration curve at concentrations of 1, 10, 25, 50, 100, 250 and 500 ng mL^{-1} using a mixture of PPCPs in ultrapure water with 0.1% formic acid: methanol (75:25). The lowest calibration point was determined to be 1 ng mL^{-1} as it is the conservative IQL. SIS mixture of 200 ng mL^{-1} of $^{13}C_2$-17α-ethynylestradiol, diclofenac-d_4,

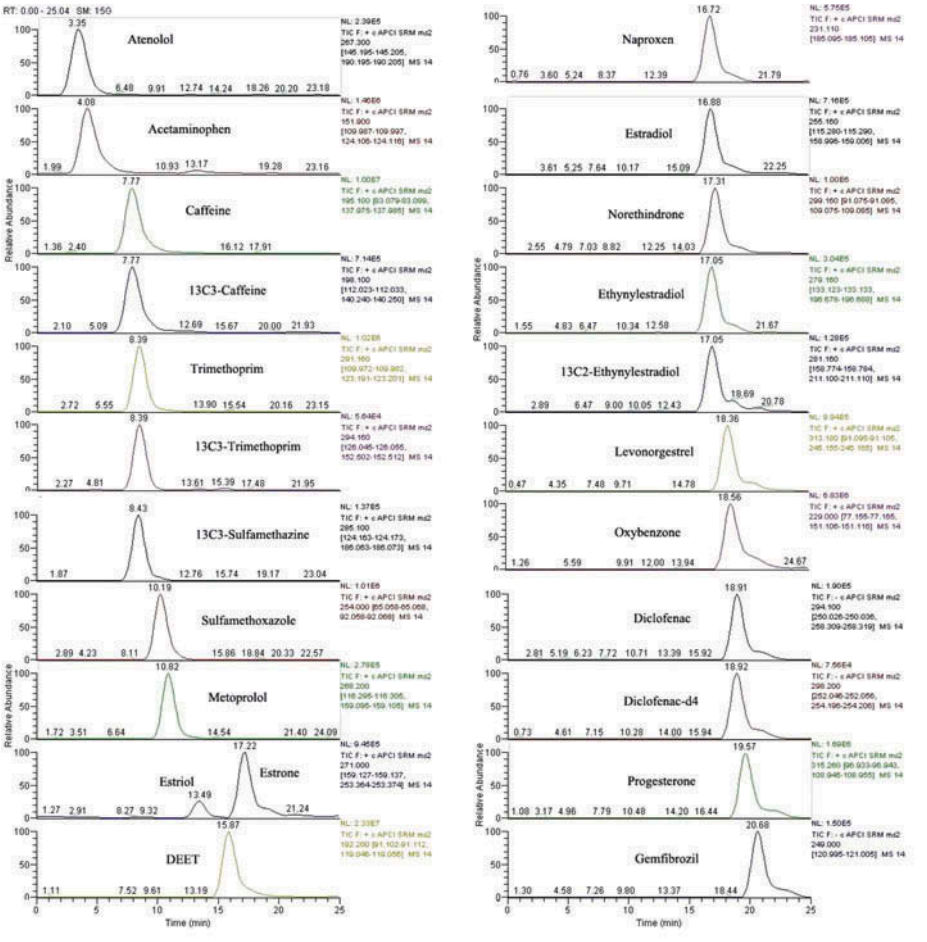

Figure 2. SRM chromatograms of PPCPs in ultrapure water with 0.1% formic acid: methanol (75:25) quantified using LC-MS/MS in APCI mode.

$^{13}C_3$-trimethoprim, $^{13}C_3$-sulfamethazine and $^{13}C_3$-caffeine were added at every calibration concentration to generate relative factor as calculated according to Equation (2). Linearity of the calibration curve was determined by employing least squares regression. The coefficient of determination (R^2) was used to determine the linearity of each target compound. Xcalibur software version 2.0.7 from Thermo Scientific (CA, USA) was used in data collection, peak integration and linear regression:

$$\text{Relative factor (RF)} = \frac{A_x C_{IS}}{A_{IS} C_x}, \tag{2}$$

where A_x is the peak area for the analyte of interest, A_{IS} the peak area for the internal standard, C_{IS} the concentration of the internal standard and C_x the concentration of the analyte of interest.

Each matrix was spiked with PPCPs mixture at concentration one to five times of estimated method detection limits (MDLs) resulting with S/N ratios between 2.5 and 5. Seven replicates of

samples were subjected to the entire methodology. MDLs were calculated based on 99% confidence level to be greater than zero and within one-third to one-fifth of the spike level using Equation (3) [32–34]. The method quantification limits (MQLs) were calculated as 10 times the standard deviation of the spike level [34]:

$$\mathrm{MDL} = t_{(n-1,1-\sigma=0.99)} \times \mathrm{SD}, \tag{3}$$

where $t_{(n-1,1-\alpha=0.99)}$ is 3.14, the Student's t-value for six degrees of freedom and SD is standard deviation of seven replicates.

SPE recoveries were validated by spiking PPCPs mixture into ultrapure water (250 ng L^{-1}), river water (250 ng L^{-1}), STP effluent (500 ng L^{-1}) and STP (1000 ng L^{-1}) influent prior to extraction. A reference sample for each matrix was spiked with the same concentration of PPCPs mixture but after SPE extraction. Percentage of SPE recovery was established by using the following equation: ($C_s/C_r \times 100\%$); percentage ratio of concentration of target compounds in spiked matrix (C_s) versus reference sample (C_r):

$$\text{Recovery } (\%) = \frac{C_s}{C_r} \times 100, \tag{4}$$

where C_s is the concentration of target compounds in spiked matrix and C_r the concentration of target compounds in reference sample.

ME were evaluated by comparing the signal of target compounds in sample matrix with the signal of target compounds in ultrapure water. River water, STP effluent and STP influent was spiked with 250, 500 and 1000, respectively after SPE extraction. ME (%) was calculated using Equation (5). as $[A_s-(A_{sp}-A_{usp})]/A_s \times 100$ where A_s is the peak area of spiked ultrapure water; A_{sp} is peak area of spiked matrix extract and A_{usp} is background concentration of matrix. ME% > 0% indicates ionisation suppression while ME% < 0% indicates ionisation enhancement. Validation of the SPE recovery and ME was performed in accordance with Al-Odaini et al. [21]:

$$\text{ME } (\%) = \frac{A_s - (A_{sp} - A_{usp})}{A_s} \times 100. \tag{5}$$

Concentrations of PPCPs in environmental matrices were calculated by using the Equation (6). Samples with concentrations out of calibration range were diluted prior to re-analysis:

$$\text{Concentration of analyte} = \frac{C_{ex} \times V_{ex}}{V_s \times \mathrm{CF}}, \tag{6}$$

where C_{ex} is concentration of target compounds in sample extract, V_{ex} the volume of sample extract, V_s the volume of sample collected and CF the concentration factor.

3. Results and discussion

3.1. *Optimisation of SPE conditions*

Many studies have featured the use of large HLB cartridges sizes such as 20 cc/1 g, 6 cc/500 mg and 6 cc/200 mg [5,8,10,32,35]. Renew and Huang [36] used an anion-exchange cartridge and HLB cartridge in tandem for the extraction of antibiotics. Other studies have used the smallest

SPE sorbent size and found acceptable recoveries of analytes [7,21]. Therefore, the smallest SPE cartridge 3 cc/60 mg was used in this method due to its lower cost and promising recovery.

Different sample volumes (25, 50, 100, 150, 200 and 250 mL) were optimised for the environmental matrices. The sample volume with the highest recovery of the PPCP standards was chosen for each matrix. The optimised sample volumes were 150 mL of river water, 150 mL of STP effluent, 100 mL of STP intermediate and 50 mL of STP influent. The optimisation of sample volume is important to avoid over-loading the SPE cartridge.

Different ratios of reconstitution solvent and formic acid were optimised to improve peak intensity and separation. It was found that the use of a higher concentration of ultrapure water in the ratio led to lower chromatogram baselines and better separation for estradiol, estriol, estrone, ethynylestradiol, levonorgestrel and norethindrone. The addition of a small amount of formic acid led to sharper peaks, better selectivity and sensitivity [37]. The optimised reconstitution solvent used in this study was 75:25 (ultrapure water with 0.1% HPLC formic acid: HPLC methanol).

3.2. *Optimisation of LC-MS/MS conditions*

3.2.1. *Optimisation of best ionisation*

Non-polar compounds such as naproxen, gemfibrozil, diclofenac, DEET and oxybenzone experienced better ionisation in APCI with higher detected peak area. Natural and synthetic hormones performed best in APCI as they experienced difficulty of ionisation in ESI mode. Similar incidences had been reported in previous literature. This could be due to their high lipophilicity and lack of functional polar groups [5,15,16]

In this study, some PPCPs such as caffeine, trimethoprim and sulfamethoxazole demonstrated higher ionisation in ESI modes. However, these minor losses in ionisation were sacrificed to achieve the main aim of unification in a single quantification. Ionisations for other compounds were comparable. APCI source was selected for further optimisation. In addition, Wang and Gardinali [16] reported lower MDLs for ibuprofen, DEET, caffeine, acetaminophen, progesterone, estradiol and ethynylestradiol for APCI compared to ESI.

3.2.2. *Optimisation of compound-dependent parameters*

Most of the precursor ions detected in APCI positive mode demonstrated protonation ($[M + H]^+$). Estriol, estradiol and ethynylestradiol each lose a molecule of water along with protonation, resulting in a $[M + H\text{-}H_2O]^+$ ion. Gemfibrozil and diclofenac were detected in APCI negative mode. Both compounds underwent deprotonation, resulting in the formation of the precursor ion $[M–H]^-$. A complete compilation of the precursor ions/product ions, tube lens and collision energy used for quantification and confirmation of PPCPs in this study is shown in Table S2 (supplemental data).

In this study, estriol and estrone experienced the same SRM transitions (*m/z* 271.0 > 253.4, *m/z* 271.0 > 159.1). To differentiate both compounds in the same SRM chromatogram, the relative abundance of their product ions was compared. The product ion of estriol (*m/z* 159.1) had a relative abundance of 31.6, while the product ion of estrone (*m/z* 159.1) had a relative abundance of 59.1. The distinct comparison between their fragmented product ions and relative abundances gave an accurate representation for trace quantification. Both compounds had different retention times, with estriol being eluted first at 13.49 and 17.22 min. Similar incidences have been reported by other researchers [15,38].

3.2.3. *Optimisation of source-dependent parameters*

Discharge current, vaporiser temperature, capillary temperature, and sheath and auxiliary gas pressure were optimised in this study. Most analytes showed a steady increase in peak area with increasing discharge current, except for estradiol, metoprolol, estrone and estriol.

Most previous studies have used a high vaporiser temperature from 350°C to 500°C [5,15]. In this study, a higher vaporiser temperature led to a decrease in peak area for most analytes, with the exception of marginal increases for metoprolol. Therefore, a vaporiser temperature of 250°C was selected. The lower vaporiser temperature in this method was expected, as a lower solvent flow rate of 100 μL min^{-1} was employed.

Meanwhile, most analytes demonstrated a major improvement at a capillary temperature of 250°C, with the most distinct improvement in norethindrone's peak area. Increasing the sheath gas pressure and auxiliary gas pressure over the range of 20–45 arb units and 5–30 arb units, respectively, yielded a steady decrease in peak areas for most PPCPs except for trimethoprim and sulfamethoxazole. The latter two demonstrated fluctuations during optimisation. The optimum sheath gas pressure and auxiliary gas pressure were 20 and 5 arb units, respectively.

3.2.4. *Optimisation of mobile phase and gradient elution*

Several mobile phases were optimised in this study, including methanol, ACN and ultrapure water, as well as different acidic additives such as acetic acid and formic acid.

Of all the optimised mobile phases and additives, ACN yielded an improved peak shape and lower baselines for chromatogram. ACN concentrations of 10%, 20% and 30% were optimised, and their impacts on the peak areas of analytes are shown in Figure S1 (supplemental data). Significantly improved peak areas of all PPCPs were found when increasing ACN from 10% to 20%, except for acetaminophen. However, a further increase of ACN (30%) was detrimental, evidencing steep reductions in all peak areas. Thereafter, 20% ACN was adopted to develop the mobile phase gradient.

Several flow rates were optimised to ensure that all 18 PPCPs had sufficient time for separation. In this study, estriol and estrone had the same precursor and daughter ions but different relative abundances. It was observed that a faster flow rate of 200 μL min^{-1} hampered adequate separation of estriol and estrone. The optimum separation of estriol and estrone was achieved at 100 μL min^{-1}.

3.3. *Analytical performance and validation*

3.3.1. *Precision*

The precision of the method was validated by repeatability (intra-day precision) and reproducibility (inter-day precision) under identical conditions. According to USEPA Method 1694, the required initial precision for acetaminophen, caffeine, gemfibrozil, naproxen, sulfamethoxazole and trimethoprim is a relative standard deviation (RSD) of not more than 30% [32]. Other studies conducted elsewhere have also revealed both intra-day and inter-day RSD values less than 15% [7,8,39,40]. In this study, the RSD values for repeatability and reproducibility ranged from 0.4% to 7.3% and 3.6% to 8.7%, respectively (Table S3 in supplemental data). Overall, the RSD for both repeatability and reproducibility was below 10%, indicating good precision.

3.3.2. *Sensitivity*

The IDLs and IQLs of PPCPs in this study were quantified in the range of 0.001–0.1 μg L^{-1} and 0.005–1.0 μg L^{-1}, respectively. The linearity of all calibration curves (R^2) was above 0.997. IDLs, IQLs and R^2 values for all PPCPs are summarised in Table S4 (supplemental data).

Table 1. MDLs and MQLs for PPCPs in various environmental matrices.

Analyte	MDL (ng L^{-1})				MQL (ng L^{-1})			
	Ultrapure water	River water	STP effluent	STP influent	Ultrapure water	River water	STP effluent	STP influent
Acetaminophen[c]	2	7	20	88	5	22	62	281
Atenolol[d]	0.1	1.5	3.5	12	0.3	3.5	10	38
Caffeine[a]	0.5	1.5	5	110.5	0.5	4	15	351.5
DEET[d]	0.5	0.5	3	36	1.5	1.5	10	113.5
Diclofenac[e]	0.2	2.5	25	55	0.5	8	78	174.5
Estradiol[b]	2	4	13	73	6.5	12	40	231
Estriol[b]	5	2.5	34	50	14	7	108	152
Estrone[b]	3	2.5	22	96.5	9	8	69	306.5
Ethynylestradiol[b]	1	4.5	19	97	3.5	13.5	60	307
Gemfibrozil[e]	2	8	10	18	5	25	32	57
Levonorgestrel[b]	3.5	2.5	30	92	10.5	7	94	292
Metoprolol[d]	0.5	2.5	7.5	39	2	8	23	123
Naproxen[d]	0.5	5	48	98	2	16	153	310.5
Norethindrone[b]	3	2.5	27	121.5	8	8.5	87	386.5
Oxybenzone[d]	2	3	5	25	5	10	16	79
Progesterone[b]	2	4.5	22	38	6	13	69	121.5
Sulfamethoxazole[c]	0.5	2.5	12.5	33	1	8	40	103
Trimethoprim[d]	0.5	2.5	5.5	15	1	7.5	17.5	47

Note: SIS: $^{13}C_3$-Caffeine[a], $^{13}C_2$Ethynylestradiol[b], $^{13}C_3$-Sulfamethazine[c], $^{13}C_3$-Trimethoprim[d], [e]Diclofenac-d_4.

The results of the MDLs of all PPCPs are depicted in Table 1. MDLs ranged from 0.1 to 5 ng L^{-1} for ultrapure water (reference material); 1.5 to 8 ng L^{-1} for river water; 3 to 48 ng L^{-1} for STP effluent and 2 to 121.5 ng L^{-1} for STP influent. PPCP quantification often involves trace analysis; therefore, low MDLs in parts-per-trillions are crucial. MDLs for acetaminophen, atenolol, diclofenac, ethynylestradiol, levonorgestrel, metoprolol and norethindrone in river water and STP effluent were lower than previously published method which quantified in ESI mode [21].

The MQLs correspond to the reporting limits of the method. Any environmental concentration below the MQL would be quantified with weak precision and poor accuracy. MQLs for each analyte are listed in Table 1. The MQLs ranged from 0.3 to 14 ng L^{-1} for ultrapure water (reference sample); 4 to 25 ng L^{-1} for river water; 10 to 153 ng L^{-1} for STP effluent and 38 to 386.5 ng L^{-1} for STP influent. MDLs and MQLs for acetaminophen and caffeine reported in this study for river water is lower than those established in USEPA method [32]. In addition, MQLs for diclofenac in STP influent and effluent is lower than previously published using ESI mode [9].

3.3.3. *Accuracy*

3.3.3.1. *SPE recovery.* SPE recovery is analyte- and matrix-specific; therefore, the percentage recovery for each PPCP was validated as shown in Table 2. The said recovery also acts as a performance evaluation for the HLB cartridge to effectively extract all target PPCPs. Recoveries for most PPCPs were above 80% with minimal exceptions. The relative recoveries of PPCPs using isotope dilution were within a range of 20–98.9% for ultrapure water (reference sample); 37–129% for river water; 54.1–96.5% for STP effluent and 63.1–97.8% for STP influent.

According to the USEPA [32], recovery for acetaminophen, caffeine, gemfibrozil, naproxen, sulfamethoxazole and trimethoprim are required to be within a range of 50–120% in reference

Table 2. SPE recovery for PPCPs spiked into ultrapure water, river water, STP effluent and STP influent at 250, 250, 500 and 1000 ng L^{-1}, respectively.

	Recovery % ± SD (n = 3)							
	Ultrapure water		River water		Effluent		Influent	
Pharmaceuticals	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
Acetaminophen	44 ± 5	35 ± 2[c]	24 ± 5	37 ± 2[c]	72 ± 18	91 ± 6[c]	93 ± 14	86 ± 7[c]
		44 ± 1[d]		39 ± 6[d]		91 ± 14[d]		86 ± 9[d]
Atenolol	9 ± 1	20 ± 2[c]	77 ± 16	98 ± 2[c]	59 ± 32	95 ± 4[c]	91 ± 5	93 ± 6[c]
		27 ± 5[d]		92 ± 7[d]		94 ± 17[d]		95 ± 4[d]
Caffeine	82 ± 1	99 ± 4[a]	44 ± 6	107 ± 3[a]	92 ± 6	93 ± 3[a]	93 ± 5	91 ± 7[a]
DEET	99 ± 4	91 ± 8[c]	94 ± 1	107 ± 7[c]	98 ± 1	97 ± 4[a]	79 ± 40	93 ± 5[a]
		94 ± 2[d]		129 ± 26[d]		95 ± 5[c]		94 ± 2[c]
Diclofenac	141 ± 26	94 ± 7[e]	93 ± 15	81 ± 3[e]	98 ± 8	91 ± 8[e]	85 ± 10	91 ± 6[e]
Estradiol	84 ± 1	71 ± 11[b]	55 ± 5	95 ± 4[b]	63 ± 3	89 ± 12[b]	75 ± 20	95 ± 3[b]
Estriol	123 ± 2	97 ± 11[b]	71 ± 8	98 ± 16[b]	84 ± 38	84 ± 13[b]	91 ± 14	89 ± 7[b]
Estrone	89 ± 15	93 ± 6[b]	78 ± 5	96 ± 2[b]	89 ± 6	72 ± 35[b]	89 ± 7	84 ± 5[b]
Ethynylestradiol	93 ± 11	90 ± 4[b]	70 ± 7	96 ± 5[b]	97 ± 10	77 ± 6[b]	87 ± 13	95 ± 4[b]
Gemfibrozil	123 ± 22	91 ± 4[e]	21 ± 2	89 ± 12[e]	48 ± 5	73 ± 7[e]	86 ± 5	63 ± 22[e]
Levonorgestrel	105 ± 9	97 ± 1[b]	97 ± 3	93 ± 5[b]	102 ± 7	91 ± 1[b]	80 ± 14	71 ± 5[b]
Metoprolol	129 ± 18	97 ± 8[c]	87 ± 13	86 ± 6[c]	96 ± 27	93 ± 9[c]	91 ± 23	95 ± 2[c]
		92 ± 8[d]		70 ± 22[d]		94 ± 1[d]		92 ± 5[d]
Naproxen	113 ± 2	93 ± 10[c]	94 ± 5	100 ± 1[c]	84 ± 8	93 ± 5[c]	88 ± 10	71 ± 18[c]
		92 ± 9[d]		91 ± 18[d]		91 ± 1[d]		64 ± 19[d]
Norethindrone	95 ± 6	90 ± 7[b]	87 ± 10	92 ± 7[b]	90 ± 33	62 ± 12[b]	89 ± 15	89 ± 2[b]
Oxybenzone	76 ± 8	96 ± 2[a]	20 ± 1	73 ± 3[a]	91 ± 26	76 ± 9[a]	91 ± 6	78 ± 14[a]
		84 ± 10[c]		44 ± 2[c]		93 ± 5[c]		75 ± 8[c]
Progesterone	102 ± 3	94 ± 6[b]	62 ± 3	125 ± 7[b]	41 ± 5	54 ± 30[b]	80 ± 7	85 ± 13[b]
Sulfamethoxazole	101 ± 2	81 ± 4[c]	77 ± 5	100 ± 5[c]	82 ± 6	86 ± 9[c]	96 ± 1	92 ± 16[c]
		86 ± 6[d]		119 ± 17[d]		77 ± 9[d]		95 ± 5[d]
Trimethoprim	144 ± 9	99 ± 4[d]	52 ± 5	98 ± 1[d]	90 ± 9	90 ± 22[d]	96 ± 3	98 ± 1[d]

Note: SIS: [a] $^{13}C_3$-Caffeine, [b] $^{13}C_2$-Ethynylestradiol, [c] $^{13}C_3$-Sulfamethazine, [d] $^{13}C_3$-Trimethoprim, [e] Diclofenac-d_4.

water when the recovery is corrected by an internal standard. The optimised method was able to fulfil this criterion for all said compounds, with the exception of acetaminophen, which had lower recoveries in the reference water and river water samples. The relative recovery for acetaminophen in reference water was 34.9% in this study, in comparison to 32%, 8.2% and 40% in previously published methods [5,21,39]. As such, the low recovery of acetaminophen was comparable with other studies.

Atenolol also experienced low SPE recovery in reference water (20.1%). The same result was observed in Lin et al. [10], where the recovery improved from 26.8% in reference water to 92.3% in river water.

Some PPCPs demonstrated a reduction in recovery as the complexity of environmental water matrices increased from river water to sewage. High organic matter and chemicals in samples will compete for binding sites, thus reducing the sorption efficiency of SPE cartridges [21,40]. This is an unavoidable phenomenon, but additional sample clean-up and more specific isotope dilution could improve the recovery of PPCPs [6,8,41].

Analyte-specific isotope dilution is recommended to overcome particularly low recovery in reference water, as well as increased matrix complexity. Vanderford and Snyder [6] demonstrated that utilising matched isotope-labelled analytes could greatly compensate SPE loss and mitigate ME. In this study, gemfibrozil and naproxen had recoveries of 63.1% and 64.1%, respectively, in STP influent. With the specific isotope dilution reported by Vanderford and Snyder [6], their recoveries were 90% and 102%, respectively. On the same note, the analytes in this study that matched the SIS compounds, that is, caffeine, ethynylestradiol, diclofenac and trimethoprim, yielded recoveries above 90% in STP influent. Therefore, isotope dilution is recommended in order to improve SPE recovery.

Despite the advantages of having exact isotope-labelled standards, this is often not practiced in most studies [7,8,10,39,40]. Isotope-labelled standards are rare and expensive. In addition, they are not available for all compounds. Common practice in the quantification of PPCPs is to provide the closest approximation to the analyte in structure and behaviour due to financial shortcomings and limited supplies.

In the process of validating the performance criteria of SPE HLB cartridges, several other parameters such as MDLs and MQLs need to be taken into consideration. According to Gros et al. [7], low recoveries of analytes are usually not an obstacle in producing reliable quantifications as long as precision (repeatability and reproducibility) and sensitivity (MDLs and MQLs) are good. All compounds demonstrated good precision and sensitivity. Therefore, the Oasis HLB 3 cc/60 mg cartridge was confirmed for further application.

3.3.3.2. *Matrix effects.* APCI mode experienced less ME especially for non-polar and steroid compounds compared to ESI mode [5,42,43]. This is beneficial when quantifying environmental matrices. The ME of each analyte is shown in Table 3. The SRM chromatograms of PPCPs and hormones spiked into different environmental matrices are shown in Figures S2 and S3, respectively (supplemental data). All target compounds demonstrated good separation with minimal noise peak.

Five SISs were added into the sample to assist in correction of recoveries for SPE and ME. The peak area of caffeine was suppressed by 203.9%, but it was recovery-corrected by SIS to an enhancement of 37.7% in STP effluent.

In the event that an exact matched SIS was not available, the most compatible SIS was evaluated and thereafter chosen based on criteria such as structural similarity, behaviour similarity and performance of recovery correction. Therefore, each analyte was matched to the four SISs spiked in this study. $^{13}C_3$-Trimethoprim was able to provide better recovery for atenolol in all three matrices. The ME after recovery correction was enhanced by 10.5% in river water, 5.3% in STP effluent and 13.2% in STP influent. A similar method for selecting SISs has been published elsewhere [7]. Lin et al. [10] adopted $^{13}C_6$-sulfamethazine as the sole SIS for quantifying 97 pharmaceuticals and hormones in environmental water samples. Another study also reported the use of 2 SISs to correct the recoveries of 28 pharmaceuticals [39]. Therefore, using five SISs in this study was deemed sufficient to provide a reasonable quantification.

Furthermore, dilutions of sample extracts were also proven to significantly reduce ME. In a study conducted by Gros et al. [7], the dilution of post-SPE extracts at ratios of 1:2 and 1:4 reduced signal suppression in STP effluent and influent, respectively. The drawback of this method is its loss in sensitivity. Another separate study concluded that post-SPE dilution requires a larger volume of SIS and resulted in slightly higher reporting limits [6]. Despite the reported drawbacks, dilution was shown to reduce ME, which is the main obstacle in environmental analysis. Instead of conducting post-SPE dilutions, which involve tedious calculations and a higher volume of SIS, which is costly, sample volume reduction was utilised in this study.

Table 3. Matrix suppression of PPCP concentrations in environmental matrices in river water, STP effluent and STP influent.

	Matrix suppression (%)					
	River water		STP effluent		STP influent	
Analyte	Absolute	Relative	Absolute	Relative	Absolute	Relative
Acetaminophen	−1.0	−37.7[c]	−10.7	−7.0[c]	−981.5	68.5[c]
		−2.6[d]		28.4[d]		−4.5[d]
Atenolol	13.2	10.9[c]	55.5	97.3[c]	55.7	65.2[c]
		−10.5[d]		−5.3[d]		−13.2[d]
Caffeine	−12.5	4.7[a]	−203.9	37.7[a]	453.5	−9.7[a]
DEET	−6.5	−4.9[c]	−118.9	17.3[c]	−18.2	26.8[c]
		4.8[d]		0.24[d]		10.6[d]
Diclofenac	29.1	−2.5[e]	3.2	29.5[e]	16.1	31.5[e]
Estradiol	−40.5	2.2[b]	−8.0	42.0[b]	44.5	41.1[b]
Estriol	−17.0	0.9[b]	−26.7	41.3[b]	89.7	13.0[b]
Estrone	−11.4	12.3[b]	32.1	−8.5[b]	−4.9	−1.3[b]
Ethynylestradiol	−24.4	−2.6[b]	−47.2	−11.0[b]	−24.8	24.4[b]
Gemfibrozil	−334.8	10.5[e]	−5.7	43.1[e]	3.9	78.2[e]
Levonorgestrel	−17.9	1.7[b]	27.6	50.9[b]	−84.0	45.0[b]
Metoprolol	−3.3	−6.6[c]	−38.1	81.6[c]	−15.8	−19.8[c]
		−1.5[d]		29.9[d]		−4.5[d]
Naproxen	15.0	2.1[c]	4.2	44.8[c]	−22.1	−4.9[c]
		5.9[d]		−0.8[d]		−12.1[d]
Norethindrone	−19.9	−0.8[b]	−10.6	37.4[b]	−102.5	68.3[b]
Oxybenzone	−32.3	−30.5[a]	127.1	88.2[a]	−10.4	78.0[a]
		−22.3[c]		95.8[c]		90.6[c]
		−5.8[d]		42.0[d]		47.9[d]
Progesterone	−57.5	−5.6[b]	4.7	46.2[b]	−9.8	46.9[b]
Sulfamethoxazole	9.7	−7.8[c]	1.0	10.7[c]	8.8	17.4[c]
		−0.4[d]		6.0[d]		19.8[d]
Trimethoprim	0.1	4.2[d]	−0.9	−0.8[d]	−6.5	9.0[d]

Note: SIS: $^{13}C_3$-Caffeine[a], $^{13}C_2$-Ethynylestradiol[b], $^{13}C_3$-Sulfamethazine[c], $^{13}C_3$-Trimethoprim[d], [e]Diclofenac-d_4.

Several sample volumes were extracted for each matrix, and their recoveries were documented and compared. The optimum sample volumes were 150 mL for river water, 100 mL for STP effluent and 50 mL for STP influent. Smaller sample volumes have been previously reported in other studies [6,21]

3.4. *Environmental application*

Limited studies have been carried out on the occurrence and distribution of PPCPs in Malaysian waters. To date, there are only a few relevant studies on the occurrence of human pharmaceuticals and synthetic hormones [9,21–23]. The occurrence of PCPs has never been studied in Malaysia. To the author's best knowledge, DEET, gemfibrozil, estradiol, estriol, estrone, naproxen, oxybenzone, progesterone, sulfamethoxazole and trimethoprim are quantified here for the first time in Malaysian waters.

Table 4. Concentrations of PPCPs in Langat River Basin.

	River water sampling stations (n = 3)					
	RW1 (ng L^{-1})		RW2 (ng L^{-1})		RW3 (ng L^{-1})	
Pharmaceuticals	Range	Mean	Range	Mean	Range	Mean
Acetaminophen[d]	77–104	93	163–448	278	148–240	195
Atenolol[d]	9–13	11	39–41	40	10–15	13
Caffeine[a]	33–63	44	210–269	238	49–60	54
DEET[d]	13–31	22	58–155	94	35–51	41
Diclofenac[e]	813–886	839	165–214	186	502–722	592
Estradiol[b]	71–402	211	174–343	281	198–263	228
Estriol[b]	444–1432	851	2557–5440	3993	1856–3059	2307
Estrone[b]	59–357	220	515–1055	785	395–963	672
Ethynylestradiol[b]	116–229	166	173–316	238	186–333	278
Gemfibrozil[e]	19–59	43	ND–44	23	52–74	60
Levonorgestrel[b]	ND	ND	61–117	91	29–44	34
Metoprolol[d]	14–25	18	84–110	96	45–68	60
Naproxen[d]	ND	ND	112–475	269	227–553	342
Norethindrone[b]	ND	ND	ND	ND	103–230	154
Oxybenzone[d]	8–10	9	8–11	10	5–8	7
Progesterone[b]	16–32	25	ND–50	25	33–90	58
Sulfamethoxazole[d]	21–10	13	31–55	50	34–54	41
Trimethoprim[d]	ND	ND	ND	ND	12–71	37

Note: SIS: $^{13}C_3$-Caffeine[a], $^{13}C_2$-Ethynylestradiol[b], $^{13}C_3$-Sulfamethazine[c], $^{13}C_3$-Trimethoprim[d], [e]Diclofenac-d_4.

The range and mean of PPCP concentrations in samples collected from the three river locations (upstream, midstream and downstream) and Extended Aeration STP (influent and effluent) are shown in Tables 4 and 5, respectively. Samples were collected in triplicate at every river water sampling point, thus totalling nine river samples for each PPCP. Meanwhile, sewage samples were collected using the ISCO 3700 Portable sampler as 48 hours time-paced multiple bottle composting. Samples were collected every 4 hours. Thus, each composite sample consisted of 12 smaller samples.

Four PPCPs, namely levonorgestrel, naproxen, norethindrone and trimethoprim, were not detected (ND) in RW1. RW1 is a recreational spot where swimming and picnic activities have previously occurred. Most PPCP peaks were concentrated at midstream, as RW2 is an urbanised town. Seven PPCPs, namely ethynylestradiol, gemfibrozil, naproxen, norethindrone, progesterone, sulfamethoxazole and trimethoprim had higher concentrations downstream at RW3.

The highest PPCP concentration detected in river water was estriol at RW2, with a mean concentration of 3993 ng L^{-1}. Norethindrone and trimethoprim were detected below MDLs in RW2. Meanwhile, gemfibrozil and progesterone were ND in RW2.

As for the concentration of PPCPs in the STP, the three highest influxes were caffeine, estriol and acetaminophen, with mean concentrations of 25,578, 7711 and 4236 ng L^{-1}, respectively. Removal efficiency of STP were calculated in accordance with Li et al [44].These compounds were all excellently removed in the STP, with a removal percentage above 85%. However, due to their high influx into the STPs, they were not completely eliminated, with significant amounts still detected in the effluent sample. The highest PPCP concentration detected in the effluent was 1000 ng L^{-1} of estriol with removal of 88.6%. A high concentration of estriol was detected in the Langat River Basin at alarming levels that could elicit chronic toxicity in aquatic organisms. According to Metcalfe et al. [45], exposure to nanogram per litre

Table 5. Concentrations of influent, intermediate and effluent and removal of PPCPs in an Intermittent Decanting Extended Aeration STP.

	Sewage treatment plants (STP) (n = 3)						
	STP 1 (extended aeration)						
	Influent (ng L^{-1})		Intermittent (ng L^{-1})		Effluent (ng L^{-1})		
Pharmaceuticals	Range	Mean	Range	Mean	Range	Mean	Removal (%)
Acetaminophen[d]	3173–4776	4236	187–275	233	73–196	115	97.3
Atenolol[d]	58–106	84	46–52	49	25–32	29	65.3
Caffeine[a]	24,438–26,499	25,578	220–250	240	103–122	115.1	99.5
DEET[d]	93–173	124	94–146	121	61–92	79	35.7
Diclofenac[e]	1867–2107	1993	1316–1852	1655	616–650	632	68.3
Estradiol[b]	841–1641	1165	719–1050	862	164–258	198	83.0
Estriol[b]	7141–8353	7711	4118–4765	4467	711–1000	883	88.6
Estrone[b]	4143–4299	4226	1095–2098	1451	342–369	357	91.6
Ethynylestradiol[b]	833–878	853	607–721	661	225–237	232	72.8
Gemfibrozil[e]	156–317	255	149–192	170	53–60	57	77.7
Levonorgestrel[b]	493–811	615	273–323	301	32–39	36	94.2
Metoprolol[d]	439–959	619	150–266	224	158–189	170	72.5
Naproxen[d]	728–935	863	276–369	330	223–224	224	74.1
Norethindrone[b]	1048–1137	1082	611–648	631	218–265	239	77.9
Oxybenzone[d]	ND–36	29	14–16	15	5–8	7	68.8
Progesterone[b]	77–91	85	42–64	57	ND–23	22	73.8
Sulfamethoxazole[c]	183–507	356	85–152	116	163–214	183	48.6
Trimethoprim[d]	28–91	55	43–69	54	8–58	30	44.8
Oxybenzone[d]	ND–36	29	14–16	15	5–8	7	68.8

Note: SIS:$^{13}C_3$-Caffeine[a], $^{13}C_2$-Ethynylestradiol[b], $^{13}C_3$-Sulfamethazine[c], $^{13}C_3$-Trimethoprim[d], [e]Diclofenac-d_4.

concentrations of estriol induces intersex (development of testis-ova) and altered sex characteristics in Japanese medaka.

High concentration of natural estrogens (estradiol, estriol and estrone) is most likely associated with discharge of untreated human and animal waste. According to Juahir et al. [46], 39% of point sources of pollution in Langat river basin consisted swine poultry. In addition, several recreational areas are non-point sources.

4. Conclusion

A selective and sensitive LC-MS/MS method was developed for the detection and quantification of 18 PPCPs in environmental waters. SPE using HLB sorbent was shown to provide an efficient method for simultaneous extraction, sample clean-up and enrichment.

High selectivity was achieved by adopting SRM mode, in which specific pairs of precursor-product ions were monitored for the purpose of quantification and confirmation. SPE loss and ME were mitigated using five deuterium-labelled SISs. Losses during sample preparation, ME and instrumental fluctuations were compensated for by the application of the SIS quantification method. The developed method was validated for its performance in ultrapure water, river water, STP effluent and STP influent. Recoveries for the majority of PPCPs were above 80% in most environmental matrices which are within acceptance level of USEPA [32]. MDLs and MQLs for some analytes in ultrapure water were as low as 0.1 and 0.3 ng L^{-1}, respectively. MDLs and MQLs for some PPCPs in river water, STP effluent and influent were lower than those reported

in USEPA and previously published methods [9,21,32]. In addition, the intra-day and inter-day precision of the quantification method were recorded to be less than 10% RSD. Thereafter, the robust and reliable method was applied for the detection of PPCPs in Malaysian environmental water.

The detection of several PPCPs, namely, sulfamethoxazole, trimethoprim, estradiol, estriol, estrone, progesterone, DEET, oxybenzone, naproxen and gemfibrozil, in Malaysian environmental waters was reported for the first time. Estriol was quantified at several sampling locations at concentrations above 1000 ng L^{-1}. These high concentrations are most probably associated to point sources pollution of untreated of human and animal wastes. Occurrences of selected PPCPs at high concentrations were alarming, indicating the possibility of eliciting aquatic toxicity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplemental data

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