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TANDEM SOLID PHASE EXTRACTION FOR THE DETERMINATION OF PHARMACEUTICALS IN WASTEWATER

(Pengekstrakan Fasa Pepejal Secara Penggabungan Katrij Untuk Analisis Farmaseutikal Dalam Air Sisa Buangan)

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Abstract

In this study, a simple and rapid tandem solid phase extraction (SPE) was developed for the analysis of seven pharmaceuticals (acetaminophen, caffeine, carbamazepine, diclofenac, naproxen, ibuprofen and metoprolol) in wastewater sample. The novel aspect of coupling SPE cartridge in tandem is the ability to simplify the SPE procedure (sample introduction step) as no single sorbent was able to retain and concentrate all selected compounds since these compounds are of different physicochemical properties. A tandem SPE cartridges using Oasis HLB and octadecyl bonded silica (C_{18}) was found to be efficient with the advantages of minimizing sample volume and reducing analysis time. Using this approach, carbamazepine, diclofenac, naproxen and metoprolol were trapped in the Oasis HLB while acetaminophen, caffeine and ibuprofen were trapped in the second cartridge (C_{18}). The instrumental limits of detection (LOD) ranged from 0.01 to 0.04 µg/L and satisfactory recoveries were obtained between 76% to 104%. The calibration curves were linear from 0.1 to 5.0 µg/mL, with correlation coefficients (R^2) in the range of 0.995 to 0.999. The developed method was applied to the analysis of pharmaceuticals in wastewater samples. The amount of pharmaceuticals detected in wastewater samples varied from 0.4 to 24.5 mg/L.

Keywords: pharmaceuticals, solid phase extraction, Oasis HLB, wastewater

Abstrak

Dalam kajian ini, pengekstrakan fasa pepejal (SPE) yang ringkas dan cepat telah dibangunkan untuk menganalisa tujuh jenis farmaseutikal (acetaminophen, kaffein, carbamazepin, diclofenak, naproxen, ibuprofen dan metoprolol) dalam sampel air sisa buangan. Aspek novel penggabungan katrij SPE secara berturutan ialah meringkaskan prosedur pengekstrakan disebabkan tiada satu penjerap yang boleh mengekstrak dan memekatkan kesemua farmaseutikal dalam satu ekstraksi kerana sifat fizikokimianya yang berbeza. Penggabungan katrij secara berturutan iaitu Oasis HLB dan silica terikat oktadesil (C_{18}) didapati lebih cekap dengan kelebihan dapat mengurangkan isipadu sampel dan tempoh masa analisis. Dengan menggunakan pendekatan ini, carbamazepin, diclofenak, naproxen dan metoprolol diperangkap dalam Oasis HLB manakala acetaminophen, kaffien dan ibuprofen diperangkap dalam katrij yang kedua (C_{18}). Had pengesanan instrumentasi yang diperolehi ialah dari 0.01-0.04 µg/L and peratus perolehan semula farmaseutikal ialah di antara 76% -104%. Graf kalibrasi yang diperolehi adalah linear dari 0.1-5.0 µg/mL,dengan nilai pemalar kolerasi (R^2) dari 0.995 -0.999. Kaedah yang dibangunkan ini telah diaplikasikan untuk menganalisa farmaseutikal dalam sampel air sisa buangan. Kepekatan farmaseutikal yang diperolehi adalah di antara 0.4 to 24.5 mg/L.

Kata kunci: farmaseutikal, pengekstrakan fasa pepejal, Oasis HLB, air sisa buangan

Introduction

From a global perspective, Malaysia has made considerable headway in environmental issues compared to other countries. In the 2010 Environmental Performance Index, Malaysia ranked 54th out of 163 examined countries and performed better than other countries like Poland, the US and Belgium in addressing environmental challenges. Rapid development and industrialization over the last few years have resulted in an increase of industrial and domestic discharges into the water system. Realizing the importance of environmental protection, the Malaysian

government once again focuses on environmental aspects in its 10th Malaysian Plan (2011-2015) called "Building an environment that enhances quality of life" that promises to introduce new legislation and incentives to ensure that all industries and consumers comply with the measures necessary to take on the country's environmental problems. The sources of water pollution can be categorized as point and non-point sources. Point sources include sewage treatment plants, manufacturing and agro-based industries and animal farms, whereas non point sources are mainly diffused sources such as agricultural activities and surface runoffs. In the year of 2010, the Department of Environment (DOE) has reported 20,348 water pollution sources comprise of sewage treatment plant (49.27%), manufacturing industries (44.57%), animal farms (3.7%) and agro-based industries (2.46%). In the recent years, worldwide focus of environmental research has widened from universal pollutants such as polycyclic aromatic hydrocarbons and pesticides to emerging pollutants such as pharmaceuticals and personal care products. Pharmaceuticals are classified as the so-called emerging contaminants which referred to previously unknown or unrecognized pollutants. Great concern towards these emerging contaminants is the fact that they may be carcinogenic, mutagenic and reproductively toxic [1]. Sewage treatment plants (STPs) are not able to remove pharmaceuticals and their excretion metabolites completely, and thus, they are discharged to different environmental compartments at concentrations ranging from μg/L to ng/L.

Commonly used pharmaceuticals selected in this study are anti-inflammatory drugs/analgesics (i.e. acetaminophen, diclofenac, and naproxen, ibuprofen), β_1 receptor blocker (i.e. metoprolol), stimulant drug (i.e. caffeine) and antiepileptic drug (i.e. carbamazepine). Sample preparation prior to the analysis of pharmaceuticals in wastewater sample requires effective, selective and rapid extraction technique to ensure optimum enrichment of the analytes of interest, as pharmaceuticals present at trace levels in wastewater compartment. Togola and Budzinski [2, 3] reported the use of octadecyl bonded silica (C_{18}), Oasis HLB and MCX as SPE cartridges for the extraction of several pharmaceuticals, whereby the use of MCX cartridge gave highest recoveries. However, MCX was able to give higher selectivity and sensitivity for basic compounds with cation exchange groups. In this study, C_{18} and HLB were chosen for the analysis of pharmaceuticals with different polarity, basicity, and acidity. Reversed phase (RP) materials (C_{18}) are clearly advantageous in terms of cost. However, its application towards polar species is restricted to weakly acidic or basic compounds. For polar compounds and strongly acidic and basic pharmaceuticals, Oasis HLB will significantly improve the extraction. The work presented here dealt with the development of simple and rapid tandem solid phase extraction (SPE) in analyzing pharmaceuticals in water. The chromatographic analysis was done using two types HPLC; HPLC with diode array detector (HPLC-DAD) and HPLC with fluorescence detector (HPLC-FLD). Fluorescent pharmaceuticals were analyzed using HPLC-FLD due to higher sensitivity and selectivity [4]. The proposed method was applied to wastewater samples to determine the occurrence these pharmaceuticals in the environment.

Materials and Methods

Reagent

Seven pharmaceuticals (as tabulated in Table 1) were purchased from respective supplier. Acetaminophen and naproxen were purchased from Sigma-Aldrich (St.Louis, USA, purity assay in range of 98-101%). Carbamazepine was obtained from Sigma-Aldrich (China, purity assay ≥ 98%). Ibuprofen was purchased from Sigma-Aldrich (Belgium, purity assay ≥ 98%). Diclofenac and metoprolol in their salt form were bought from Sigma-Aldrich (Steinheim, Italy, purity assay ≥ 98%). Methanol, acetonitrile of HPLC-grade and ultrapure water were purchased from Merck (Darmstafd, Germany). Phosphate buffer solution was prepared from phosphate buffer tablet obtained from CALBIOCHEM, Germany. SPE cartridges, packed with 1 g of C18 and 60 mg of Oasis HLB were purchased from Biotage (Europe) and Waters (Milford, MA, USA). Ultrapure water purchased from Merck (Darmstafd, Germany) was used to prepare the spiked water sample.

Instrumentation

HPLC-DAD was performed on Agilent 1200 Series (Germany) equipped with diode array detector 3 using Acclaim Polar Advantage2 (C_{18} column), 150 mm x 4.6 mm internal diameter with 5 μm particles. For fluorescent compounds, separation was carried out on Agilent 1200 Series (Germany) with fluorescence detector using ZORBAX Rx-SIL column, 250 mm x 4.6 mm internal diameter with 5 μm particles.

Chromatographic Conditions

Chromatographic analysis of acetaminophen and caffeine, methanol and water were used as mobile phases and detection were done at 254 nm and 280 nm, respectively. Diclofenac and naproxen were analysed using acetonitrile and phosphate buffer adjusted to pH 3 with detection at 235 nm and 280 nm. For carbamazepine, acetonitrile and water was used as mobile phases and detected at 280 nm. All analysis was done at 40 °C and flow rate of 1.0 mL/min. For chromatographic analysis of ibuprofen, mobile phase was acetonitrile (30):25 mM phosphate buffer (70), flow rate of 1.2 mL/min, excitation wavelength at 263 nm, and emission wavelength at 288 nm. Separation of metoprolol was achieved using acetonitrile (70):25 mM phosphate buffer (30), flow rate of 1.0 mL/min, excitation and emission wavelength at 229 nm and at 298 nm, respectively. The retention time registered for all pharmaceuticals is tabulated in Table 1.

Preparation of Standard Solution

A stock solution of 1000 mg/L for each pharmaceutical was prepared by dissolving standard pharmaceuticals using methanol (HPLC-grade), ethyl acetate: acetone (50:50, v/v), and deionized water. The standard solutions were prepared for calibration purposes by diluting the stock solution in the range of 0.01 to 50 mg/L.

Solid Phase Extraction (SPE)

The target pharmaceuticals were extracted from water samples using two types of SPE cartridges (C_{18} and Oasis HLB). The pharmaceuticals (2 mg/L and 3 mg/L) were spiked using ultrapure water for the recovery test. The elution procedure for C_{18} was adapted from the study conducted by Togola and Budzinski [3]. The extraction procedures were improved by connecting SPE cartridges (C_{18} and Oasis HLB) in tandem arrangement with Oasis HLB on top of that C_{18}. The conditioning step was done separately, whereby C_{18} is conditioned using 3 ml of 50:50 (ethyl acetate: acetone), 3 mL of methanol and 3 ml of deionized water. For Oasis HLB, conditioning was done by 3 mL of methanol, followed by 3 mL of deionized water. Water sample (500 mL) was loaded onto the tandem cartridges at 15 mL/min. After sample loading, cartridges were separated and let to dry for 30 minutes. Washing of cartridges was done using 6 ml deionized water and 6 mL of methanol and once again were let to dry for 1 hour. Elution of pharmaceuticals from C_{18} and Oasis HLB was done using 9 ml of 50:50 (ethyl acetate: acetone) and 6 mL of methanol, respectively. Pharmaceuticals obtained from each cartridge were evaporated to 1 mL prior to chromatographic analysis.

Method Validation

In order to apply the proposed method to environmental samples, the instrumental method was validated based on several parameters as follows:

Linearity

Ten different low level concentrations (0.1-5.0 μg/mL) of each pharmaceutical were chosen for linearity studies. The responses were measured as peak areas versus concentration.

Precision

The injection of repeatability was determined by five replicates of each standard pharmaceutical. The precision of the method was established by five replicates of the spiked water sample.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established by injection of seven replicates of very low concentration for each pharmaceutical

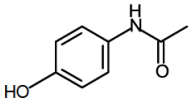
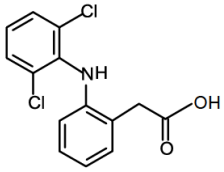
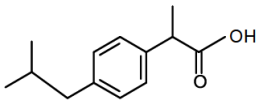
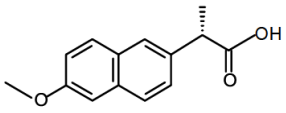
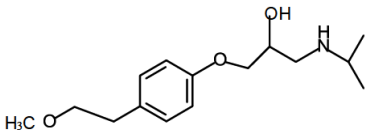
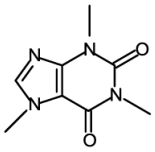
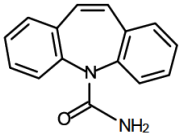
Preparation of Spiked Sample

Distilled water (100 mL) was adjusted to pH 2.0 and filtered through 0.45 μm glass fibre filter (Whatman International Ltd Maidstone, England). Sample was then spiked with the working standard solution of seven pharmaceuticals (3 μg/mL). Spiked sample was agitated for 1-2 min prior to SPE extraction.

Preparation of Water Sample

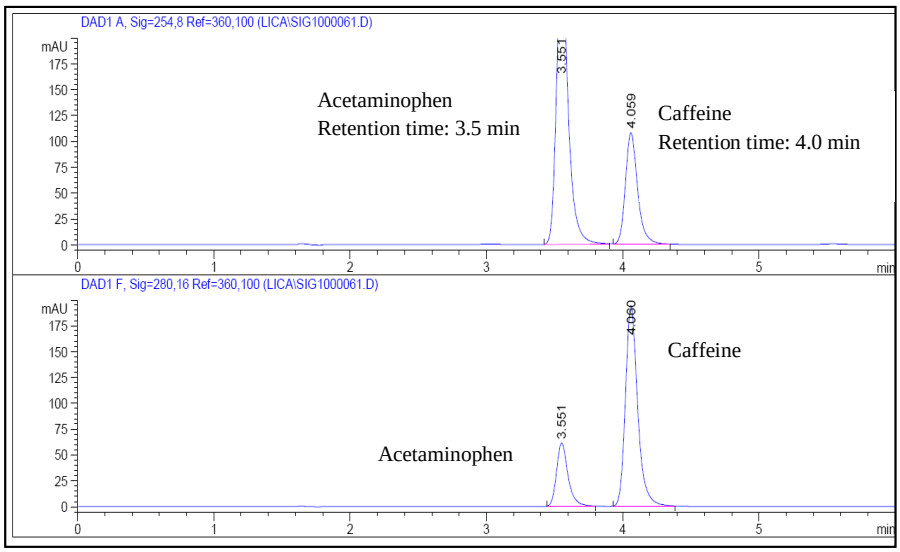
Wastewater samples obtained from wastewater treatment plant (WWTP) of Indah Water Consortium and Universiti Teknologi MARA (UiTM, Shah Alam, Selangor) were filtered through 0.45 μm glass fiber filter to remove suspended matter. Samples were stored in amber bottles at 4 °C until analysed.

Table 1. Studied pharmaceuticals with various parameters (therapeutic group, chemical properties, structure, molecular weight, pKa and retention time).

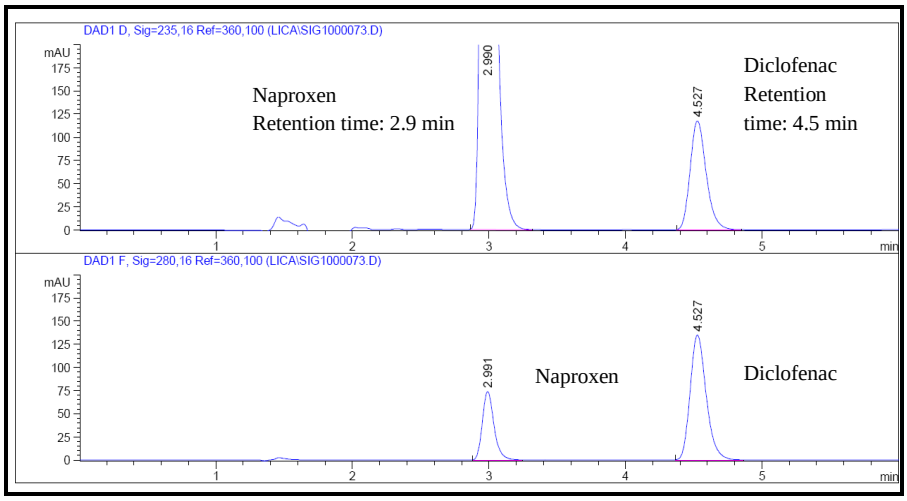
Pharmaceuticals	Therapeutic group	Chemical properties	Structure	MW (g/mol)	pKa	Retention Time
Acetaminophen	Anti-inflammatory/ analgesics	Acidic		151	9.5	3.2-3.4
Diclofenac	Anti-inflammatory/ analgesics	Acidic		194	14.0	3.9-4.0
Ibuprofen	Anti-inflammatory/ analgesics	Acidic		236	13.4	2.7
Naproxen	Anti-inflammatory/ analgesics	Acidic		295	4.0	4.0-4.1
Metoprolol	β1receptor blocker	Basic		230	4.4	2.9
Caffeine	Stimulant	Basic		206	4.2	1.8
Carbamazepine	Antiepileptic drug	Basic		267	9.68	5.4

Data of DIC, IBU, NAP, MET and CBZ from [5], Data of ACT and CAF from [6]

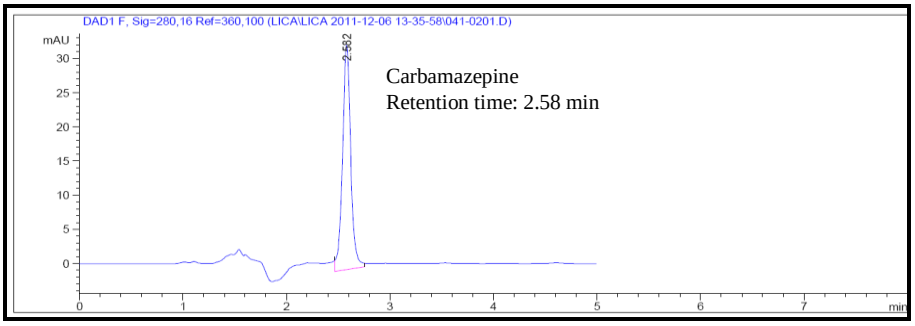
Figure 1 shows the chromatogram of standard pharmaceuticals analysed using HPLC-DAD at wavelength of 235 nm, 254 nm and 280 nm.



(a)



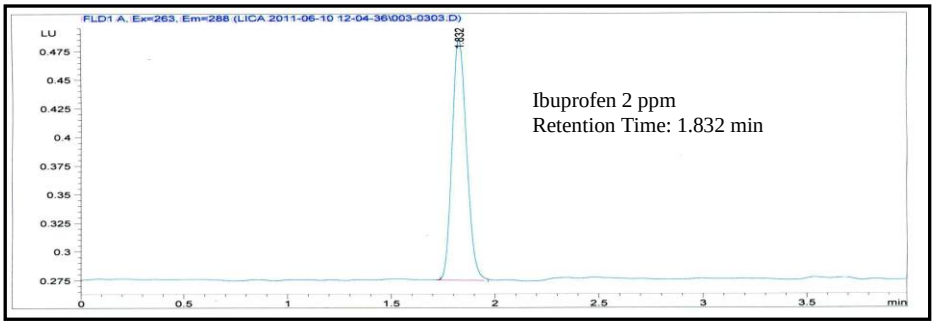
(b)



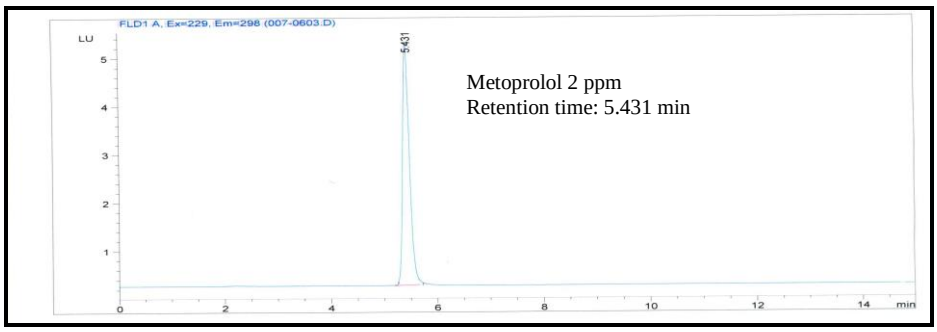
(c)

Figure 1. Chromatogram of standard pharmaceuticals separated using HPLC-DAD; (a) Acetaminophen at 254 nm and ibuprofen at 280 nm; (b) Naproxen at 235 nm and diclofenac at 280 nm; (c) Carbamazepine at 280nm.

Figure 2 (a) and (b) show the chromatogram of standard ibuprofen and metoprolol separated using HPLC-FLD.



(a)



(b)

Figure 2. Chromatogram of standard ibuprofen and metoprolol using HPLC-FLD.

Results and Discussion

HPLC Separation

In this study, two types of liquid chromatography were used to analyse seven pharmaceuticals; HPLC-DAD and HPLC-FLD. HPLC-DAD was used to separate caffeine, acetaminophen, diclofenac, naproxen, and carbamazepine, while HPLC-FLD was used to separate ibuprofen and metoprolol. Selection of wavelengths for the detection of these compounds was based on previous studies [7, 8]. Column temperature for HPLC-DAD was set at 40 °C in order to increase column efficiency and decrease retention time. Common solvents; methanol, acetonitrile (ACN), phosphate buffer and deionized water were used as mobile phases. However, due to the different physicochemical properties of these compounds, separation was done separately using various compositions of mobile phases. Acetaminophen and caffeine were separated using deionized water: methanol with 70:30 ratios, while diclofenac and naproxen was separated using ACN: phosphate buffer (pH 3) with 70:30 ratio. Separation of carbamazepine was achieved using ACN: deionized water with 60:40 ratios. Ibuprofen and metoprolol was separated using ACN and phosphate buffer (pH 7.4) with ratio of 30:70 and 70:30, respectively.

Linearity study was done using individual standard or standard mixtures with concentration ranges from 0.1 - 5.0 µg m/L. In order to justify the applicability of the proposed method, calibration curve was prepared using the least square regression analysis between average area and concentration of pharmaceuticals. Linear relationship of the correlation coefficients (R^2) in the range of 0.995 to 0.999 was obtained for all studied pharmaceuticals (Table 2). The linearity of the calibration curves gave an indication on the suitability of the chromatographic method over a wide range of concentration for the studied pharmaceuticals. The repeatability of the HPLC injection system as well as the developed method is determined by the values of the relative standard deviation for five replicates injection of the standard solution of the studied pharmaceuticals. The relative standard deviation (RSD) is presented in Table 2. Low RSD (< 1%) was obtained for all pharmaceuticals, showing good precision.

The instrumental LOD and LOQ were determined and expressed as µg/L, varied from 0.007 to 0.054 and the LOQ was determined from the calculated LOD, ranged from 0.023 to 0.374. This low value of LOD and LOQ shows the method is applicable to be used for the analysis of pharmaceuticals in wastewater sample. Considering the sample enrichment (0.5 L water sample will be reconcentrated to 1000 µL) obtained and environmental concentrations reported for the studied pharmaceuticals, we can propose the application of this HPLC method to study the occurrence of pharmaceuticals in the water compartments as it enable the detection of studied pharmaceuticals at trace levels (ng/mL)

Table 2. Method Validation Parameters

Pharmaceuticals	Retention Time (min)	LOD (µg/L)	LOQ (µg/L)	Precision (% RSD)	Coefficient Correlation, R^2
Acetaminophen	3.4	0.013	0.041	0.025	0.999
Caffeine	4.0	0.013	0.041	0.025	0.999
Carbamazepine	2.7	0.037	0.374	0.159	0.998
Diclofenac	4.0	0.007	0.023	0.136	0.999
Ibuprofen	1.8	0.054	0.161	0.027	0.998
Naproxen	2.8	0.007	0.023	0.099	0.995
Metoprolol	5.4	0.021	0.067	0.525	0.999

Arrangement of Tandem Cartridges

The capability of SPE cartridges was evaluated using water sample spiked with the working standard solution of seven pharmaceuticals (3 μg/mL). Since no single sorbent was able to retain and concentrate compounds with different physicochemical properties, preliminary study was conducted to evaluate the performance of C_{18} and Oasis HLB on the retention of the selected pharmaceuticals. C_{18} was chosen as it is advantageous in terms of cost, although its application towards polar species is restricted to weakly acidic or basic compounds. Oasis HLB is used for the extraction of polar compounds and strongly acidic and basic pharmaceuticals to overcome the limitation of C_{18}. Retention mechanism of both C_{18} and Oasis HLB is based on reversed phase [9]. However, Oasis HLB is more specific to hydrophobic compounds with some hydrophilic functionality, especially aromatics (lipophilic divinylbenzene). Hydrophilic N-vinylpyrrolidone provides retention of polar analyte. Carbamazepine, diclofenac, naproxen and metoprolol were efficiently trapped in the Oasis HLB (Table 3), while acetaminophen, caffeine and ibuprofen were trapped in C_{18}. Based on these results, tandem SPE cartridges approach was developed. Positioning Oasis HLB on top of $C_{18,}$ specific pharmaceuticals (carbamazepine, diclofenac, naproxen and metoprolol) were retained by Oasis HLB, while the pharmaceuticals (acetaminophen, caffeine and ibuprofen) poorly retained by Oasis HLB will pass through and retain by the second cartridge, C_{18}. Retention mechanism of C_{18} is based on the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface. However, if the positioning of C_{18} is above Oasis HLB, only some of the hydrophobic compounds will be trapped, while other will pass through. This resulted in poor recoveries of some pharmaceuticals (carbamazepine, diclofenac, naproxen and metoprolol). The proposed tandem SPE approach with Oasis HLB on top of C_{18} successfully retained seven pharmaceuticals selected in this study as tabulated in Table 3. Satisfactory and comparable recoveries of pharmaceuticals were obtained using both single cartridge and arrangement in tandem approaches.

Table 3. Recoveries (%) of pharmaceuticals using different approach of SPE extraction.

Pharmaceuticals	Single Cartridge (n=3)		Tandem Cartridges (C_{18}-Oasis HLB) (n=3)	
	C_{18}	Oasis HLB	C_{18}	Oasis HLB
Acetaminophen	94.5 ± 0.9	nd	90.5 ± 1.5	nd
Caffeine	104.6 ± 4.1	nd	87.4 ± 15	nd
Ibuprofen	60.4 ± 3.3	nd	76.6 ± 1.6	nd
Carbamazepine	nd	95.7 ± 0.1	nd	89.0 ± 1.4
Diclofenac	nd	88.9 ± 0.6	nd	104.7 ± 0.1
Naproxen	nd	102.3 ± 3.6	nd	90.2 ± 3.5
Metoprolol	nd	89.1 ± 2.0	nd	85.3 ± 2.0

nd = not detected (below the limit of detection)

Acetaminophen gave good recovery (90.5%) using the tandem cartridges approach as compared to single cartridge Oasis HLB approach reported by Justin *et al.* [10] with 52.8% recovery. The tandem cartridges approach also gives good recovery of caffeine (87.4%) as compared to that (67.7%) obtained by Martin *et al.* [1]. Improved recovery of ibuprofen was obtained using tandem SPE cartridges as compared to single cartridge approach, and the recovery of this pharmaceutical (60.4%-76.6%) concurred with previous studies conducted by Azzouz *et. al* and Yu & Wu [4,8]. Recoveries of carbamazepine, diclofenac, naproxen and metoprolol using Oasis HLB single cartridge were in the range of 88.9%-102.3%, comparable to the recoveries obtained using tandem SPE cartridges approach (85.3%-104.7%). This result showed that the use of tandem cartridges can significantly simplify the extraction procedures by using one-step sample loading for seven pharmaceuticals with different physicochemical properties, thus reducing the analysis time with an advantage of minimising sample volume.

Analysis of wastewater samples

The developed method was applied for the analysis of pharmaceuticals in wastewater samples from Indah Water treatment plant and Universiti Teknologi Mara (UiTM) treatment plant in Shah Alam, Selangor. Table 4 shows the amount of pharmaceuticals detected in samples from these wastewater treatment plants. Acetaminophen and caffeine were found in high concentrations in influent wastewater sample obtained from UiTM (10.7 mg/L and 24.5 mg/L), respectively compared to wastewater sample from Indah Water (8.5 mg/L and 11.3 mg/L), respectively. The results showed high consumption of these pharmaceuticals among the students' community. Ibuprofen and naproxen were found in higher concentration from wastewater sample of Indah Water (3.4 mg/L and 1.5 mg/L) compared to water sample of UiTM (0.7 mg/L and 0.43 mg/L), respectively. Concentrations of 1.5 µg/L for naproxen and 85 µg/L for ibuprofen have been found in some STP effluents [6] and low removal efficiencies (between 15% and 69%) for diclofenac [11] have been reported by several researchers. The concentration of diclofenac from the wastewater sample of Indah Water was 2.0 mg/L, but was not detected in wastewater sample of UiTM as this pharmaceutical is used for reducing inflammation and as analgesic reducing pain for arthritis or acute injury, which may not be commonly used by the students. Prolonged exposure to diclofenac has been reported to cause toxic effects and bioaccumulation in fish [12]. The concentration of carbamazepine was below LOD in both wastewater treatment plant samples. Metoprolol, used for the treatment of hypertension, angina pectoris and arrhythmia was detected from the wastewater sample of UiTM (1.2 mg/L), but not from the wastewater sample of Indah Water. The reported concentrations of all pharmaceuticals were significantly higher and not in the same order with those reported in European countries [6, 9, 13]. However, significant amounts of these pharmaceuticals were detected from water samples of both treatment plants suggested that they can be good chemical markers for human contamination and may be a very promising alternative for verification of consumption of pharmaceuticals in local communities.

Table 4. Concentrations of the pharmaceuticals in the WWTP of Indah Water and UiTM.

Pharmaceuticals	Concentration of Pharmaceuticals (mg/L), n=3	
	UiTM	Indah Water
Acetaminophen	10.7 ± 1.0	8.5 ± 0.8
Caffeine	24.5 ± 1.5	11.3 ± 0.2
Ibuprofen	0.7 ± 2.6	3.4 ± 4.0
Naproxen	0.4 ± 2.6	1.5 ± 1.2
Diclofenac	nd	2.0 ± 4.0
Carbamazepine	nd	nd
Metoprolol	1.2 ± 0.02	nd

nd = not detected (below the limit of detection)

Conclusion

A rapid and selective (SPE) using tandem-cartridges Oasis HLB and octadecyl, C_{18} for the determination of seven pharmaceuticals in wastewater sample was developed. The Oasis HLB sorbent retained the polar compounds and strongly acidic and basic pharmaceuticals (carbamazepine, diclofenac, naproxen and metoprolol), while the weakly acidic or basic compounds (acetaminophen, caffeine and ibuprofen) were poorly retained in Oasis HLB will pass through and retained by the C_{18}. The developed SPE procedure using tandem SPE cartridges approach of Oasis HLB and C_{18} was found to be efficient with the advantage of minimizing sample volume and reducing analysis time. This developed tandem SPE cartridges approach has allowed simultaneous extraction using two different sorbents in a single sample loading. The application of the developed method on wastewater sample from two treatment plants was able to detect the significant amount of the studied pharmaceuticals with acceptable repeatability. The amount of pharmaceuticals in wastewater may be applied in the estimation of the usage of pharmaceuticals in a community.

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