Journal of Chromatography A, 1345 (2014) 139–153

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Multi-residue analytical methodology-based liquid chromatography-time-of-flight-mass spectrometry for the analysis of pharmaceutical residues in surface water and effluents from sewage treatment plants and hospitals

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ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 4 April 2014
Accepted 9 April 2014
Available online 18 April 2014

Keywords:
Pharmaceuticals
Solid phase extraction
Effluent and influent of hospital
Sewage treatment plants
Multi-residue analytical method
LC–TOF/MS

ABSTRACT

An analytical method that facilitated the analysis of 11 pharmaceuticals residue (caffeine, prazosin, enalapril, carbamazepine, nifedipine, levonorgestrel, simvastatin, hydrochlorothiazide, gliclazide, diclofenac-Na, and mefenamic acid) with a single pre-treatment protocol was developed. The proposed method included an isolation and concentration procedure using solid phase extraction (Oasis HLB), a separation step using high-performance liquid chromatography, and a detection procedure that applies time-of-flight mass spectrometry. The method was validated for drinking water (DW), surface water (SW), sewage treatment plant (STP) influent and effluent, and hospital (HSP) influent and effluent. The limits of quantification were as low as 0.4, 1.6, 5, 3, 2.2 and 11 ng/L in DW, SW, HSP influent and effluent, STP effluent, and STP influent, respectively. On average, good recoveries higher than 75% were obtained for most of the target analytes in all matrices. Matrix effect was evaluated for all samples matrices. The proposed method successfully determined and quantified the target compounds in raw and treated wastewater of four STPs and three hospitals in Malaysia, as well as in two SW sites. The results showed that a number of the studied compounds pose moderate to high persistency in sewage treatment effluents as well as in the recipient rivers, namely; caffeine, simvastatin, and hydrochlorothiazide. Ten out of 11 compounds were detected and quantified in 13 sampling points. Caffeine was detected with the highest level, with concentrations reaching up to 9099 ng/L in STP influent.

1. Introduction

Pharmaceuticals are a group of emerging, potentially hazardous contaminants that have attracted increasing concern in recent years. These compounds are considered one of the most emerging environmental issues. Pharmaceuticals are found in surface water (SW), sewage treatment plant (STP) influent and effluent, and in the hospital (HSP) influent and effluent at levels reaching up to a few micrograms per liter [1–4]. Pharmaceutical residues are introduced into the aquatic environment from numerous sources such as insufficiently treated sewage effluents, production residues, improper disposal of expired medications and unused drugs, land fill leachates, and accidental spillage during manufacturing and distribution [5]. Pharmaceuticals exist in low concentrations in the environment (down to a few nanograms per liter), so that highly sensitive and selective analytical methods are required for detection. Various methods have been reported in the literature in which LC–MS, LC–MS/MS, or LC–QqLIT–MS hybrid triple quadrupole-linear ion trap mass spectrometer [6–9] were utilized instead of GC–MS [10–12] because of the often laborious derivatization

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http://dx.doi.org/10.1016/j.chroma.2014.04.025
0021-9673/

Table 1
Physico-chemical properties of all compounds [15–18].

Compound (therapeutic class)	PKa	Log K_{ow}	M(wt/(g/mol))	Solubility (mg/L)	Consumption (kg/yr)
Caffeine (stimulant)	10.4	−0.07	194.190	2.16×10^4	NA[a]
Prazosin (antihypertensive)	6.5	1.28	383.41	310	107
Enalpril maleate (renin-angiotensin)	3.0	0.49	392.53	0.025	555.9
Carbamazepine (antiepileptics)	13.9	2.45	236.28	17.7	NA[b]
Nifedipine (calcium channel blockers)	<1	2.20	346.34	56.3	3124
Levonorgestrel (sex hormones)	NA	3.48	312.46	2.05	2.6263
Simvastatin (lipid modifying agents)	13.49	4.68	418.58	0.03	1223.5
Hydrochlorothiazide (diuretics)	7.9	−0.07	297.739	722	295.5
Gliclazide (diabetics)	NA	2.12	323.412	52.1	3905
Diclofenac-Na (anti inflammatory)	4.2	0.70	318.149	2430	3661.6
Mefenamic acid (anti inflammatory)	4.2	5.12	241.2851	20	31577.1

[a] Over the counter (OTC), not prescribed.
[b] Not available with top 40 pharmaceuticals in Malaysia 2007.

procedures required prior to the analysis with GC–MS to increase the volatility of the analytes. A combination of quadrupole and TOF–MS (Q-TOF) coupled to LC is a powerful tool for the identification of trace compounds of complex mixtures and/or for confirming the presence of these compounds [1,13]. The occurrence of human pharmaceuticals and synthetic hormones in the Malaysian aquatic environment has never been reported except for a few studies on SW and STP effluent [4,14]. The lack of data could be attributed to the absence of a standard method for the analysis of pharmaceutical residues in water matrices. Considering the limited information, the present study aims to develop an accurate, sensitive, and selective multi-residue analysis for the simultaneous determination of 11 pharmaceuticals with different therapeutic classes of interest in environmental matrices, such as SW, HSP influent and effluent, and STP influent and effluent, using LC–ESI–TOF/MS. The authors cover different types of water matrices to collect more information on the occurrence of pharmaceuticals in Malaysia. In our study, only 11 pharmaceutical standards and three internal isotope standards were investigated because of the high cost of obtaining a large number of pharmaceutical standards and isotope standards.

The pharmaceuticals listed in Table 1 and Fig. 1 were selected on the basis of their consumption in Malaysia [15], environmental occurrence, and persistency, as reported in previous studies [1,6,10,13,14]. The method is based on a single solid phase extraction (SPE) protocol for different sample volumes, followed by LC–ESI–TOF/MS instrument analysis with 16.1 and 15.1 min total run time for positive (PI) and negative ion (NI) modes, respectively. Several key points such as optimization of elution program and mobile phase to improve the separation and enhancement of signal-to-noise (S/N) ratio were discussed. Moreover, optimization of elution solvent to enhance the extraction of target analytes was also performed.

2. Experimental

2.1. Chemicals and materials

Pure standards (≥98%) of nifedipine (NFD) (CAS no. 21829-25-4), enalapril maleate (ENL) (CAS no. 76095-16-4), prazosin (PRZ) (CAS no. 19237-84-4), caffeine (CAF) (CAS no. 58-08-2), levonorgestrel (LNG) (CAS no. 797-63-7), carbamazepine (CBZ) (CAS no. 298-46-4), simvastatin (SMV) (CAS no. 79902-63-9), hydrochlorothiazide (HDZ) (CAS no. 58-93-5), gliclazide (GLZ) (CAS no. 21187-98-4), diclofenac-Na (DIC-Na) (CAS no. 15307-79-6), and mefenamic acid (MEF) (CAS no. 61-68-7) were purchased from Sigma-Aldrich (USA). The isotopically labeled compound used as surrogate and internal standard, CAF-$^{13}C_3$ (CAS no. 603295 Fluka), was also purchased from Sigma-Aldrich (USA). SMV-D_6 (CAS no. S485002) and DIC-D_4 were purchased from Toronto Research Chemicals (Canada). Deionized water (DIW) used was supplied by EASYPure RODI (USA). HPLC-grade methanol (MeOH), acetonitrile (ACN), acetone, and formic acid (FA) were supplied by Merck (Germany). Methyl tertiary butyl ether (MTBE) was supplied by Baker (USA).

The cartridges used for SPE were Oasis HLB (3cc, Waters, USA), SUPELCLEAN ENVI-CHROM P (6cc, Supelco, USA) SUPELCLEAN LC-SAX (3cc, Supelco, USA) and Oasis MCX (3cc, Waters. USA).

Individual stock standard solutions (1000 mg/L) were prepared in HPLC-grade MeOH and stored at −18 °C to minimize the degradation of the standard. A mixture of all pharmaceutical standards was prepared by appropriate dilution of the individual stock solutions. Further dilutions of this mixture were prepared in MeOH–DIW (1:9, v/v) before each analytical run and were used as the working standard solutions. Prior to use, all glassware were boiled with water at 100 °C, rinsed with distilled water, dried in an oven at 200 °C for 2 h, subsequently rinsed with MeOH, and dried in the oven at 200 °C for 2 h.

2.2. Sample collection

All samplings were performed in Negeri Sembilan, one of Malaysian's 13 states, which lies on the western coast of Peninsular Malaysia, immediately south of Kuala Lumpur and borders Selangor on the north, with Pahang in the east and Malacca and Johor to the south. The population in Negeri Sembilan is approximately 1.0 million, and the total area is approximately 6686 km^2. Four STP, three HSP, and two recipient SW (Langat and Muar rivers) samples were collected from the state. A total of 13 whole sampling points in the present study included the SW as well as the influents and effluents of STPs and HSPs as presented in Fig. 2.

Table 2 shows the influent and effluent wastewaters (WWs) were collected from four STPs (STP1, STP2, STP3, and STP4) and three HSPs (HSP1, HSP2, and HSP3). Samples were also collected from the recipient rivers (RRs) at two points (RR1 and RR2). The first three STPs (STP1, STP2, and STP3) are the main sources of sewage effluent in the River Langat, and STP4 is considered the biggest source in the Negeri Sembilan. Sampling was conducted in May, June and July 2013. All samples were collected in 1 L amber glass bottles with Teflon-lined caps to ensure sample integrity using a high density polyethylene plastic (HDPE) bucket previously rinsed with distilled water and MeOH. The bottle head space was kept to a minimum by completely filling the bottles. The bottles were rinsed in the field twice with the sample and completely filled on the third sampling. Disposable gloves were used by the sampler to prevent any personal care products from contaminating the sample bottles.

2.3. SPE

Samples were preserved by adding 1 g/L of sodium azide to prevent microbial degradation. Aliquots of 100, 250, 500, and 1000 mL