Online SPE-UPLC-MS/MS for herbicides and pharmaceuticals compounds' determination in water environment: A case study in France and Cambodia

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ABSTRACT

This study assessed the environmental footprint of emerging micropollutants in Cambodia and France. The aim was to develop and apply an analytical method to detect micropollutants in diverse water sources and climatic regions. Consequently, an analytical method, using online solid-phase extraction coupled with an ultra-performance liquid chromatography-tandem mass spectrometer (online-SPE-UPLC-MS/MS), was successfully developed and validated. This method permits the accurate and rapid multi-residual determination of 15 emerging micropollutants in water at low detection and quantification limits, around 10 ng.L^{-1} and 30 ng.L^{-1}, respectively, within a total analytical run of seven minutes, including the equilibrium step. The findings revealed that no water body was free of micropollutants in any case of its sources (effluent wastewater, surface water, and even tap water). In surface water, 13 and 11 of the 15 target micropollutants were detected at least once in the Couesnon River (France) and Upper Mekong River (Cambodia), respectively. The concentration of micro-pollutant detected in Couesnon River ranged from 6–975.5 ng.L^{-1}, with tramadol having the highest concentration. In the Upper Mekong River, the concentration detected ranged from 5–240 ng.L^{-1}, with ketoprofen having the highest concentration. Caffeine was found in the highest concentration in the treated effluent of a Cambodian wastewater treatment plant (WWTP).

Introduction

Pesticides are used globally to boost the yield of agricultural products, and prescription medicines are consumed in large quantities and used on a daily basis to prevent and treat human diseases. These products contain active compounds, which are usually persistent or metabolized in the environment when disposed of. They reach water bodies through many routes, for example, herbicide runoff from agricultural land to surface and groundwater (Margoum et al., 2006; Vallée et al., 2014). Moreover, pharmaceutical discharges occur into sewers via human excretion, animal farms, and through the disposal of expired medicine. The prevalence of chemicals such as pesticides, metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and phthalates in fresh water in mainland France has been reported by the Ecology Ministry since 2003 (Dubois and Lacouture, 2011). Later, the presence of micropollutants was continuously discovered in surface water, groundwater, and even in potable water in France (Togola and

Budzinski., 2008; Hladik et al., 2008; Mompelat et al., 2011; Vulliet and Cren-Olivé, 2011; Petit and Michon, 2016). It was observed that the concentration of emerging contaminants was higher than the allowable standard. Thus, continuous monitoring measures need to be implemented. For example, the concentration of chloroacetanilide herbicides and their metabolites was reported to be above 0.1 µ.L^{-1} (Hladik et al., 2008). The concentration of pesticides above 0.1 µg.L^{-1} in drinking water leads to a failure in complying with the regulations as laid out in the drinking water directive for single compound pesticides (Directive 98/83/CE, 1998). Many studies have been conducted in France, whereas the amount of data recorded pertaining to micropollutants in Cambodia has been limited. Additionally, a few pharmaceutical compounds were detected in trace concentration in the river (Doung et al., 2010).

Although the concentrations of these micropollutants were detected in trace quantities at low levels (ng.L^{-1} to µg.L^{-1}), they may lead to adverse health effects on humans and other non-target organisms if a continuous inflow of such pollutants is allowed to seep into the aquatic

environment. Thus, numerous studies have been conducted, and many analytical methodologies for determining herbicides and pharmaceuticals in various water matrices have been developed. Typically, the analytical tool used for detection is either gas chromatography or liquid chromatography (LC), followed by mass spectrometry (MS) (Ferrer et al., 2001; Cahill et al., 2004) or tandem MS (Gros et al., 2006; Cimetiere et al., 2014; Kachhawaha et al., 2020). The preferred method for determining trace concentrations of herbicides and pharmaceuticals in water environment is LC-MS/MS since it provides an enhancement in terms of versatility and simplified sample preparation over GC–MS. The derivatization step can be avoided, and low detection limits (LODs) can still be achieved even at concentrations of 1 ng.L^{-1}, as reported by Farré et al. (2007).

To improve the detection quality of low or trace concentrations of micropollutants in water environment, pre-concentration by solid-phase extraction (SPE) of a water sample is mandatory before analysis. However, there are many disadvantages associated with the conventional SPE method. It is time consuming, requiring a large amount of sample. Moreover, increasing error sources from multiple manual steps of extraction (Lindholm-Lehto et al., 2015; Xue et al., 2015). However, an alternative approach, online-SPE, which is an automated extraction step coupled directly to LC-MS, has gained increasing popularity and proven successful for pharmaceutical analysis. Online SPE methods used to detect emerging micropollutants in aquatic environments have been reported. Most of these methods studied only one class of compounds, such as pharmaceutical (Lindberg et al., 2014; Camilleri et al., 2015; Bazus et al., 2016; Pérez-Lemus et al., 2022) or pesticides (Jansson et al., 2010; Postigo et al., 2010). However, only a few methods that include pharmaceuticals, pesticides, and their metabolites using online SPE-UPLC-MS/MS have been documented (Huntscha et al., 2012; Togola et al., 2014).

The method for simultaneous determination of pesticides and pharmaceutical compounds in surface water, groundwater, and wastewater was commonly done in France but it is up to date for Cambodia's water. Different climatic regions and the development status of a country can contribute to the presence of micropollutants. Therefore, this study aimed to develop a rapid online-SPE-UPLC-MS/MS method for determining 15 compounds of herbicides and pharmaceuticals classes. The method was used to determine the target compounds in the treatment step of a water treatment plant in France (Couesnon River, pre-treated water) and Cambodia (wastewater effluent, Upper Mekong River, and drinking water). In addition, the matrix effect was also investigated, and the standard addition method was used to quantify the pharmaceutical and herbicide compounds in the different water samples.

Materials and methods

Chemicals of interest and reagents

The compounds were selected using screening indicators, such as the chosen molecules (i) should have a wide range of physical properties, e.g., functional group(s) and polarity; (ii) should be from a variety of pharmaceutical and herbicide classes; and (iii) should have a high frequency of environmental occurrence and have poor removal efficiencies by wastewater and drinking water treatment plants in France and other countries. Fifteen molecules, such as chloroacetanilide herbicides and their metabolites, nonsteroidal anti-inflammatory drugs, antiepileptic drugs, and stimulants, were selected for this study. The properties of each target molecule, such as the formula, molecular weight, log Kow, log D, pKa, and solubility, are presented in Table S1.

The UPLC-MS grade solvent used as the mobile phase had a purity of 98%. Sigma Aldrich (France) supplied acetonitrile (98%), methanol (98%), and formic acid (99%). An Elga PURELAB system generated the ultra-pure water (UPW) used for this study (resistivity 18.2 MΩ.cm^{-1}, TOC< 50 μg C.L^{-1}). All of the concentrated stock solutions of individual herbicides and pharmaceuticals were prepared by dissolving the pure

products in methanol to attain a concentration of 100 mg.L^{-1}. The stock solution was stored in dark at -20°C. The concentrate mix solution was prepared by diluting an individual stock solution in methanol to reach 5 mg.L^{-1} and stored at -20°C for a maximum of 15 days. The diluted mix solution was prepared daily by diluting the concentrate mix solution in water to obtain 100 μg.L^{-1}. This solution was used to spike the sample prior to conducting the analysis. The standard addition method was achieved by performing analyses of the non-spiked and spiked samples, where 4–6 spiking levels were selected according to the expected target concentration (among 1, 5, 10, 25, 50, 75, 100, 250, 500, 1000, 1500, and 2000 ng.L^{-1}).

Sampling sites location

The water samples were collected from the drinking water treatment plant (DWTP) in Mézières-Sur-Couesnon (France) and Chroy Chongva DWTP in Phnom Penh (Cambodia). The different samples were collected along the French DWTP: (a) raw water, Couesnon River, (b) pre-treated water, after the coagulation–flocculation process (see details in Figure S1). The water samples from Cambodia were accordingly analyzed: (c) treated wastewater (WW), meaning water released into the environment, (d) Upper Mekong River water source of the Chroy Chongva DWTP (Phnom Penh, Cambodia), and (e) produced drinking water (Figure S2). The samples were filtered with 0.2 μm and stored in a cold room at 4°C. A maximum of 20 ml of filtered sample was required for a single analysis in online-SPE-UPLC-MS/MS.

Online solid-phase extraction (online-SPE) sample extraction

Online extraction was performed using a 2777 autosampler (Waters), outfitted with two parallel Oasis^{TM} HLB cartridges (Direct connect HP 20 μm, 2.1 mm x 30 mm) that worked in an alternating sequence. The samples were placed in a 32-position vial holder of 20 ml. The 20 mL vials were filled with spiked and non-spiked samples. Before injecting the sample, the injection system (syringe, injection port, and sample loop) was flushed with UPW/MeOH (50:50) and UPW. The online pre-concentration process began when a sample was loaded through the injection port. The loading pump (QSM) was set at 2 mL.min^{-1}, with 100% UPW pushing the sample from the 5 mL injection loop through the extraction column 1. Once the target analytes were trapped, the weakly retained interferences were washed away. After this washing step, the analytes trapped on the SPE 1 cartridge were eluted with the elution gradient, produced by the binary solvent manager (BSM) pump onto the UPLC analysis chromatographic column. During this step, the SPE 2 cartridge was regenerated and then rebalanced under the initial conditions for the following analysis (see Figures S3 and S4). Two six-position Everflow^{TM} valves were used to switch from the loading flow pattern to elution, conditioning, and back to loading. A quaternary pump (Acquity^{TM} QSM) supplied the loading eluent (UPW) and conditioning eluent (methanol). The analytes were eluted from the SPE cartridge to the UPLC system by connecting the cartridge to the separation column's inlet and using the initial chromatographic elution solution.

UPLC-MS/MS

The UPLC-MS/MS analysis was performed on a Waters® (Acquity UPLC) liquid chromatographic system equipped with a mass spectrometer detector (Quattro premier, Micromass^{TM}). The mass spectrometer was run with the following conditions: cone gas (N$_2$, 50 L.h^{-1}, and 120°C), dissolved gas (N$_2$, 750 L.h^{-1}, 350°C), collision gas (Ar, 0.1 mL. min^{-1}), and capillary voltage (3000 V). Chromatographic separation was performed using the Waters BSM pump equipped with a vacuum degasser and a thermostatted column oven set at 45°C. A reversed-phase column (Acquity^{TM} BEH C18, 100 mm x 2.1 mm, ID, 1.7 μm) was also used. The eluents for the BSM pump were 0.1% formic acid in acetonitrile and 0.1% formic acid in UPW. The elution gradient was produced

and optimized as described in the results section.

Method validation

The linearity, precision, limit of detection (LOD), and limit of quantification (LOQ) in UPW and raw water from the Mézières-sur-Couesnon DWTP were evaluated to validate the developed analytical method. The linearity of response was evaluated by using a 12-point calibration curve, with concentrations ranging from 1 ng.L^{-1} to 2000 ng.L^{-1} and coefficient of determination (r^2). The precision or repeatability of analysis was determined by injecting the samples spiked at 10 ng.L^{-1}, 100 ng.L^{-1}, and 1000 ng.L^{-1} in a row and calculating the relative standard deviation (RSD) of six injections. The LOD and LOQ were calculated according to equations 1 and 2 and following the AFNOR NF-T-90-210 standards for all the analysts.

$$LOD = \frac{b + 3\sigma_b}{a} \qquad (1)$$

$$LOQ = \frac{b + 10\sigma_b}{a} \qquad (2)$$

where a, b, and σ$_b$ represent the slope of the calibration curve, the intercept, and the standard deviation on the intercept, respectively.

Results and discussion

Mass spectrometry optimization

The first step of method development was infusion, i.e., direct introduction of a standard diluted solution at 5 mg.L^{-1} of an individual target compound into the mass spectrometer to identify the source and fragment ions and create a multiple reaction monitoring (MRM) transition method library. To do so, the electrospray ionization (ESI) source of the mass spectrometer was used in the positive mode according to the compound's structure. The negative mode was also evaluated to ionize the target molecules. The expected best results were obtained using ESI+ (positive mode), allowing the efficient ionizing of all compounds, whereby running the analysis in both the positive (+) and negative (-) modes was avoided. The fragmented cone voltage and collision energy were optimized for each individual compound by infusion. As the parent ion, the pseudo-molecule ion [M+H]$^+$ was chosen to determine the mass-to-charge (m/z) ratio. All studied compounds give at least two transitions. The highest intensity serves as quantification and the other as confirmation. The optimum results for the detected parameters, such as cone voltage, collision energy, and ionization mode, for each compound are presented in Table 1.

Chromatographic optimization conditions for LC-MS/MS

To improve the separation and sensitivity of the method, parameters such as intensity, peak area, peak shape, and retention time, affecting both chromatographic analysis and MS/MS detection, were studied. A water/acetonitrile gradient acidified with formic acid was used in UPLC, with the BEH C18 column at the mobile phase flow rate of 0.4 mL.min^{-1}. This flow rate was the optimum zone of the Van Deemter curve with the BEH C18 column (Van De Steene and Lambert, 2008). The addition of 0.1% formic acid improves sensitivity over both the acetic and ammonium formats (Axel et al., 2017). Water and acetonitrile were preferred as the basic components of the mobile phase since they exhibit lower column pressure and provide better resolution than methanol (Perrin et al., 2002). The method permitted the separation of 15 compounds within a total analytical run of seven minutes, including the equilibrium step. The global retention (SPE + analytical separation) was mainly driven by the polarity of the compound. The plotting of polarity (log D) of the compounds is presented in Figure 1. Among the 15 compounds, the less retained or polar compounds were caffeine (log D=0.28), atenolol (log D=-1.85), and acetaminophen (log D=0.40), whereas the more retained or non-polar compounds were diclofenac, alachlor, and metolachlor, exhibiting a log D higher than 2. This indicated that the peak separation was satisfactory. As confirmation, a previous study by Bazus et al. (2016) found that the reversed-phase HPLC column (BEH C18 and HSST3) provides a satisfactory separation with k' ranging from 0.93 to 9.91, according to the polarity of the considered compounds. In addition, based on the compound's polarity, trial-and-error tests of the mobile phase gradients were applied to obtain a good peak separation. As a result, the mobile phase was initially started with 80% water and 20% acetonitrile. Its mobile phase gradient was obtained as shown in Figure 1. An example of chromatographs achieved with a solution of 100 ng.L^{-1} for the 15 target compounds in UPW can be found in Figure S5. To obtain a good chromatogram, in multi-compounds detection, the MS dwell time has to be considered. Dwell time has a significant impact on the quality of the mass spectra because lower t$_{Dwell}$ results in more noise on the baseline and peak (Gross, 2017).

As a result, the dwell time was varied between 50 and 100 milliseconds depending on the number of MRM transitions monitored concurrently. For a good peak shape and reproducible peak evolution, 10 data points per peak are required (Gross, 2017). This was defined as a requirement for adequate chromatographic peak coverage, as shown in the results in Table 1.

Table 1

List of compounds with precursor and product ions, fragmented voltage, collision energy, and retention time used for the MRM method.

Target names and identification code	Precursor (m/z)	P. ion* (m/z)	Cone (V)	col* (V)	P.ion* (m/z)	Cone (V)	col* (V)	t$_{Dwell}$ (ms)	DPP*	RT*	r^2
Diuron (DIU)	233.0	71.8 [a]	25.0	18.0	159.8	25.0	25.0	50	17	3.56	0.9997
Alachlor (ALA)	270.3	68.3	23.0	19.5	238.3 [a]	23.0	10.5	100	28	4.66	0.9985
Metazachlor (MATA)	278.3	133.9 [a]	16.5	20.5	210.1	16.5	10.0	100	11	3.80	0.9994
Metazachlor OA (Meta OA)	273.6	133.9 [a]	12.5	18.5	161.9	12.5	10.5	100	50	2.02	0.9998
Metazachlor ESA (Meta ESA)	324.1	133.9 [a]	20.0	27.0	256.1	20.0	12.0	100	40	1.82	0.9994
Metolachlor (METO)	284.0	176.1	25.0	25.5	252.2 [a]	25.0	15.5	100	22	4.68	0.9955
Metolachlor OA (Meto OA)	280.3	148.3	25.0	23.5	248.1 [a]	25.0	14.0	100	55	3.30	0.9999
Metolachlor ESA (Meto ESA)	330.3	202.1	23.0	26.5	298.2 [a]	23.0	14.5	100	13	2.15	0.9998
Acetaminophen (PARA)	151.9	92.6	24.5	20.5	109.9 [a]	24.5	15.5	50	16	1.35	0.9977
Diclofenac (DICL)	296.2	214.9[a]	24.0	18.5	250.1	24.0	12.5	100	24	4.33	0.9992
Ketoprofen (KETO)	255.1	104.7	25.0	23.5	209.1 [a]	25.0	14.0	50	14	3.66	0.9991
Carbamazepine (CBZ)	237.2	193.9 [a]	25.0	17.5	194.0	25.0	29.5	50	20	3.03	0.9994
Tramadol (TRAM)	264.5	120.9	25.0	25.0	246.3 [a]	25.0	11.0	50	12	1.68	0.9961
Caffeine (CAF)	195.1	109.8	20.0	24.5	137.9 [a]	20.0	19.5	50	11	1.41	0.9968
Atenolol (ATE)	267.2	73.8	14.5	22.5	144.8 [a]	14.5	24.5	50	12	1.33	0.9985

*Remarks: P.Ion = product ion, Col = collisions, DPP= data peak point, RT= retention time

[a] quantitative ion