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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 micropollutant 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

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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.

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

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

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

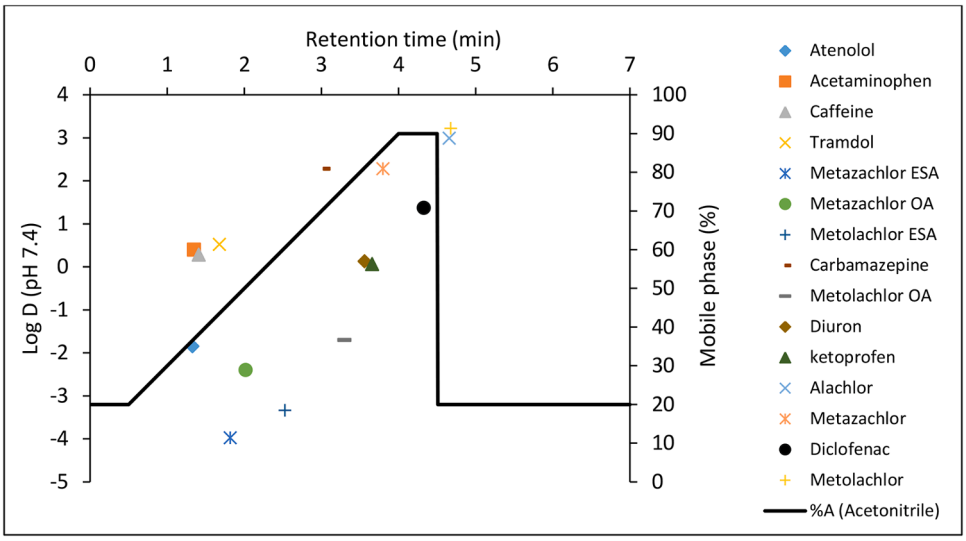


Fig. 1. Plotting of polarity (log D) versus retention time of the compound and mobile phase gradient

Method validation

The developed method was validated by regression linearity, precision, the limit of quantification, and the observed matrix effect. For the initial evaluation, UPW was used to avoid interference from organic species. The results of precision, LOD, and LOQ of each target

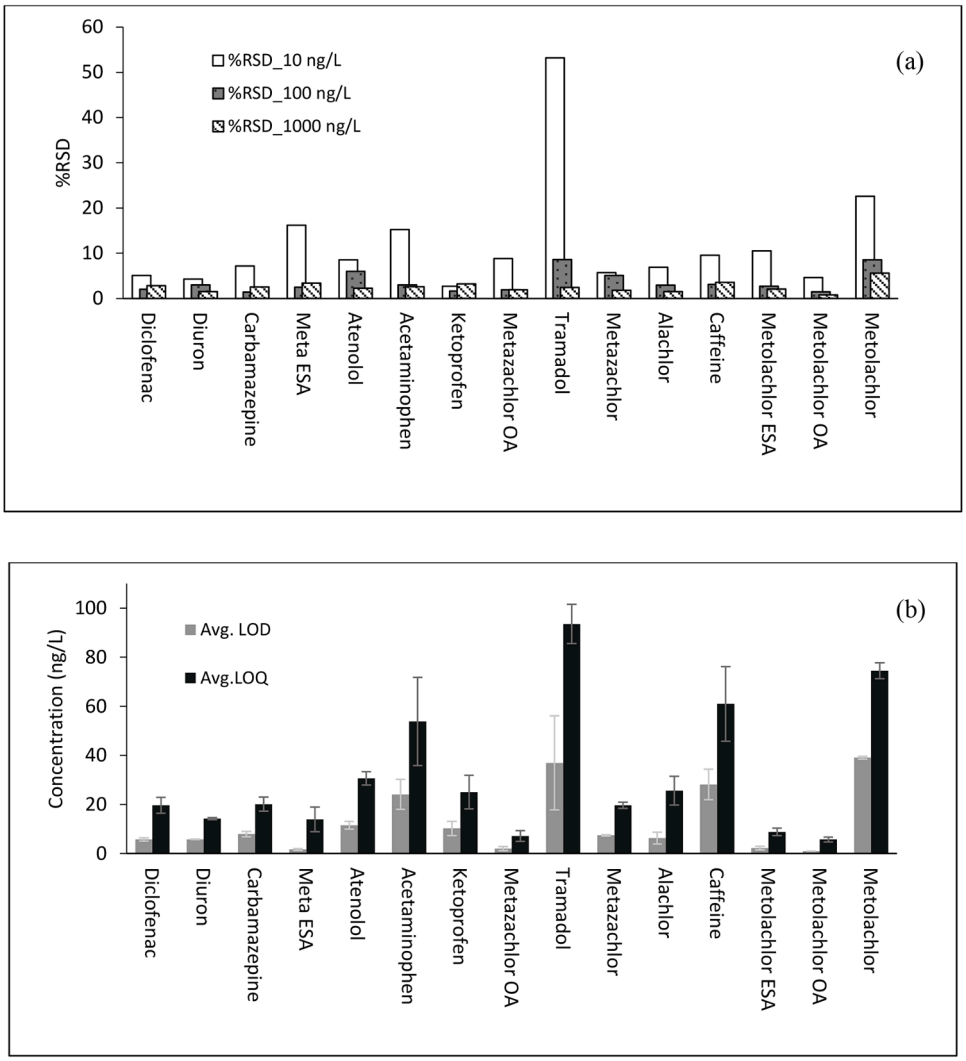


Fig. 2. (a) precision; (b) LOD and LOQ of each target molecule

compound, including the overall evaluation of the method, are illustrated in Figure 2. The method demonstrated excellent linear regression across the calibration range, with an r^2 value greater than 0.99 for all the target compounds (Table 1). In addition, spiking blank samples (UPW) at three different concentration levels (10, 100, and 1000 ng.L^{-1}) were analyzed in six replications and used to assess the analytical procedure's overall precision. The results showed that at a lower concentration of 10 ng.L^{-1}, the single compound's overall relative standard deviation percentage (%RSD) was an average of 12%, whereas some substances such as metazachlor ESA, acetaminophen, tramadol, and metolachlor showed higher than average values, with %RSD of 16%, 15%, 53%, and 23%, respectively. However, at concentrations of 100 ng.L^{-1} and 1000 ng.L^{-1}, the method was precise. The % RSD was an average of 4%, and 3% for concentration at 100 ng.L^{-1} and 1000 ng.L^{-1}, respectively. In addition, the highest %RSD was only 8% (tramadol, metolachlor) and 5% (metolachlor), corresponding to the concentrations of 100 ng.L^{-1} and 1000 ng.L^{-1}, respectively.

The linearity of the method was evaluated within the range of 1 to 500 ng.L^{-1}, and the LOD and LOQ were determined by using the statistical interpretation of the calibration curve. The LOD and LOQ of the target compounds were mainly below the average values of 13 ng.L^{-1} and 32 ng.L^{-1}, respectively, except for acetaminophen, tramadol, caffeine, and metolachlor. The lowest LOD and LOQ values were 1 ng.L^{-1} and 6 ng.L^{-1}, corresponding to the metabolite of chloroacetanilide herbicides (metolachlor OA, metolachlor ESA, metazachlor ESA, and metazachlor ESA) while the highest LOD and LOQ values were found for tramadol at 36 ± 19.2 ng.L^{-1} and 93 ± 8.0 ng.L^{-1}, respectively. The acceptable LOQ must be in accordance with the EU directive for drinking water (Ljujic and Sundac,1998). This LOQ objective was fixed at 100 ng.L^{-1}. Thus, the method was fully validated.

The correlation of %RSD, LOD, LOQ, linearity (r^2), dwell time, and data point per peak (DPP) were analyzed by using the statistical Pearson correlation. The results can be found in Table S2. There was a negative correlation between LOD and LOQ versus linearity (r^2), with the correlation coefficient of -0.964 ($p<0.01$) and -0.969 ($p<0.01$), respectively. The best linearity provides the lowest LOD and LOQ values while poor linearity leads to higher LOD and LOQ values. Another correlation between precision (%RSD) versus LOD and LOQ was observed. The coefficient correlation was 0.756 ($p<0.01$) and 0.766 ($p<0.01$), respectively, indicating that a higher %RSD will also increase the values of LOD and LOQ. The DPP and optimal dwell time also influenced each other with a correlation of 0.570 ($p<0.05$).

Matrix effects observation

The matrix effect is one of the crucial factors influencing the trueness of LC-MS/MS methods. It is caused by the presence of coeluting matrix compounds, which affect the analyte's ionization and cause significant quantification errors if not adequately corrected or minimized. Many approaches have been developed in environmental analysis to compensate for such systematic errors due to matrix effects, such as internal calibration, external calibration, external sample calibration (matrix-matched calibration), and standard addition method. If isotopically labeled standards are unavailable, the standard addition method can be considered because it provides precise results and appears to be a quantification method to compensate for matrix effects in highly loaded samples (Stüber and Reemtsma, 2004). Thus, the standard addition calibration method was used to avoid the matrix effect in this study. The standard addition curve was conducted for two water samples taken from Couesnon River and pre-treated water at the DWTP. The matrix effect was observed directly through the plot of linear regression of MS response versus the analyte concentration in UPW and the Couesnon River and pre-treated water sample, as shown in Figure 3. The contribution of the matrix effect was determined by the ratio of the slope of Couesnon river, pre-treated water's standard addition curve over the slope of standard curve prepared in UPW.

The slope ratio of the Couesnon River and pre-treated water is illustrated in Figure 4. A slope ratio of 1 indicates no matrix effect. The matrix effect does, however, interfere for values greater or lower than 1. For this study, a 90% confidence interval was used to determine the compound having a matrix effect. Among the 15 target compounds only diclofenac, diuron, carbamazepine, metazachlor, alachlor, and metolachlor could be considered as being unaffected by the matrix effect for the Couesnon River samples, whereas only diclofenac and tramadol did not exhibit the matrix effect in pre-treated water. The content of total organic matter was 6 ± 0.1 mg.L^{-1} and 3 ± 0.3 mg.L^{-1} for Couesnon River and pre-treated water, respectively. Both water bodies had similar pH (~8) and turbidity (22–29 NTU) values. However, the UV absorbance values at 254 nm of Couesnon River and pre-treated water were 0.36 cm^{-1} and 0.18 cm^{-1}, respectively. Signal suppression was generally observed for all water types analyzed, except for carbamazepine, metazachlor, and alachlor. As expected, signal suppression was significantly higher in the surface water sample than in the other sample types (Figure 4). This effect could probably be attributed to the surface water initially containing those target compounds. In general, polar compounds that were more retained experienced greater signal suppression than non-polar compounds that were less retained in all types of water. This finding was in accordance with other studies that have employed

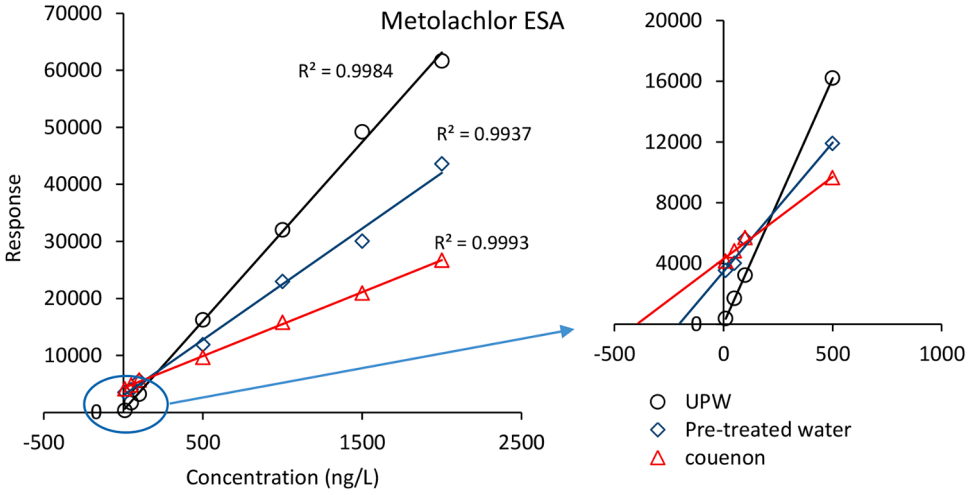


Fig. 3. Plot of linear regression in UPW, Couesnon River, and pre-treated water samples

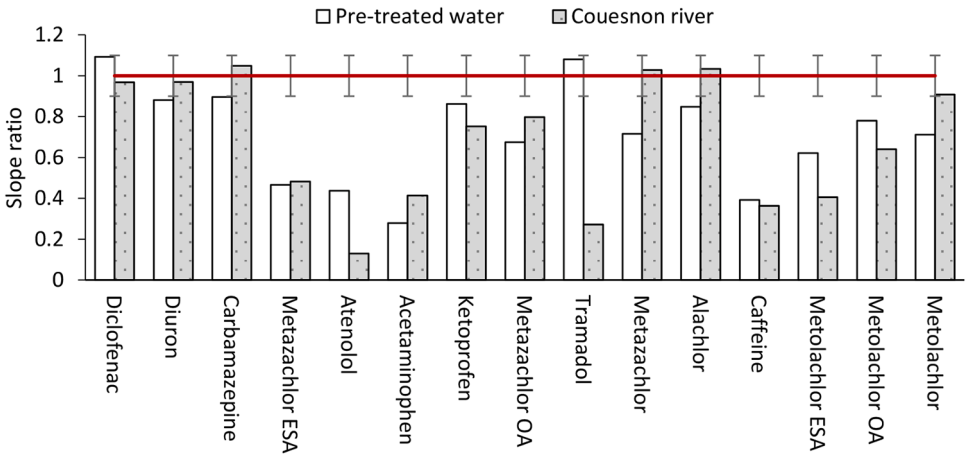


Fig. 4. MS response slope ratio in Couesnon River and pre-treated water

UPLC-MS/MS, where other analysts (Bazus et al., 2016; Cimetiere et al., 2013), such as pharmaceuticals, were determined in surface water.

Application of analytical methods

The applicability of the method was assessed by analyzing the samples taken from France: (a) the Couesnon River, (b) pre-treated water and the sample taken from the Cambodian study area, such as effluent WWTP, (c) Upper Mekong river, and (d) drinking water. Among the 15 target micropollutants, 86% could be detected in at least one sample. The results of micropollutant detection of each sample are shown in Table 2. A summary of the results per sample is provided in Table 3.

In France, the site was representative of the type of agriculture pressure. High concentrations of certain pesticides in water (metolachlor metabolites) were specifically observed in this area, even though those pesticides were banned in France long time ago (Petit and Michon, 2016). For the French water samples, acetaminophen and atenolol were not detected. A total of 13 compounds were detected in Couesnon surface water; however, the compound that had concentrations higher than LOQ were diuron, metazachlor OA, metazachlor ESA, metolachlor, metolachlor OA, metolachlor ESA, diclofenac, carbamazepine, tramadol, and caffeine. As reported by previous studies, similar contaminants were also found in water treatment plants in France (Bazus et al., 2016; Vulliet and Cren-Olivé, 2011; Hladik et al., 2008). In this study, a compound with concentrations greater than 100 ng.L^{-1}, partially removed after the coagulation treatment process, was also observed.

Table 3
Summary of the quantification results from the analysis of different samples

Sample	Number of compounds detected >LOD (relative to 15)	Number of compounds quantified > LOQ (relative to 15)	Concentration Range (ng.L^{-1})	Substance with the highest concentration
Couesnon river	13 (86.67%)	10 (66.67%)	6.0-975.4	Tramadol
Pre-treated water	9 (60%)	2 (13.33%)	3.0-403.5	Tramadol
Effluent WWTP	9 (60%)	9 (60%)	14.0-3176.2	Caffeine
Upper Mekong River	11 (73.33%)	8 (53.33%)	5.0-240.0	Ketoprofen
Phnom Penh Drinking water	1 (6.67%)	1 (6.67%)	58.0	Caffeine

Tramadol, metolachlor ESA, caffeine, and metolachlor OA were quantified in the Couesnon River (a) with concentrations 975 ± 110.7, 190 ± 2.7, and 181 ± 9.6, 33 ± 0.3 ng.L^{-1}, respectively. However, this data shows that following pre-treatment by coagulation–flocculation, caffeine was no longer detected in the pre-treated sample, (b) while

Table 2
Mean concentration of the target compounds quantified in the different matrix samples in Cambodia and France

Class	MPs	France		Cambodia				
		Surface water (ng.L^{-1}) Feb-2020	Pre-treated water (ng.L^{-1}) Feb-2020	Effluent WW (ng.L^{-1}) Feb-2020	Sep-2021	Surface water (ng.L^{-1}) Feb-2020	Sep-2021	Drinking water (ng.L^{-1}) Feb-2020
Herbicides	DIU	22 ± 1.7	< LOQ	443 ± 0.6	90 ± 0.3	< LOQ	< LOQ	< LOQ
	ALA	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	16 ± 1.2	< LOQ
	META	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	20 ± 0.4	< LOQ
	META OA	6 ± 0.5	< LOQ	< LOQ	6 ± 1.9	< LOQ	5 ± 1.0	< LOQ
	META ESA	28 ± 1.3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	METO	53 ± 4.7	< LOQ	185 ± 2.3	< LOQ	< LOQ	52 ± 1.1	< LOQ
	METO OA	33 ± 0.3	12± 0.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	METO ESA	190 ± 2.7	166 ± 3	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Anti-inflammatory	PARA	< LOQ	< LOQ	< LOQ	103 ± 5.1	< LOQ	56 ± 3.3	< LOQ
	DICL	23 ± 1.1	< LOQ	332 ± 5.3	280 ± 11.0	< LOQ	67 ± 1.8	< LOQ
	KETO	< LOQ	< LOQ	< LOQ	234 ± 6.9	< LOQ	240 ± 6.8	< LOQ
Anti-epileptic	CBZ	18 ± 0.7	< LOQ	35 ± 0.8	< LOQ	< LOQ	< LOQ	< LOQ
pain reliever	TRAM	975 ± 110.6	403 ± 33.0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Psychostimulant	CAF	181 ± 9.5	< LOQ	3175 ± 133.4	< LOQ	58± 15.0	53 ± 4.8	58 ± 3.7
B-blocker	ATE	< LOQ	< LOQ	18 ± 2.3	132 ± 0.3	< LOQ	< LOQ	< LOQ

<LOQ = Lower than the limit of quantification

tramadol (403± 33.0 ng.L^{-1}), metolachlor ESA (166 ± 3.2 ng.L^{-1}), and metolachlor OA (12 ± 0.1 ng.L^{-1}) were still detected in higher proportions than the set quality standard. This observation indicated that coagulation was ineffective in eliminating those substances, especially chloroacetanilide herbicides and their metabolites; for example, the removal efficiency of metolachlor ESA was just 12.8%.

The grab samples from the Cambodian water bodies were collected with seasonal differences: rainy season (May to September) and dry season (October to April). For the discharge into the river of the wastewater plant (c) six compounds were detected. Caffeine, diuron, diclofenac, metolachlor, carbamazepine, and atenolol were detected during the dry season while the other compounds, such as ketoprofen, acetaminophen, and metazachlor OA, were detected during the rainy season. It was observed that the highest concentration detected was caffeine, with an average concentration of 3175 ±133.4 ng.L^{-1} in the dry season. The observed changes with the change in season indicated that caffeine, diuron (443 ± 0.6 ng.L^{-1}), diclofenac (332 ± 5.3 ng.L^{-1}), metolachlor (185 ± 2.3 ng.L^{-1}), and carbamazepine (35 ± 0.8 ng.L^{-1}) were detected at higher concentrations for the water sampled in the dry season.

In contrast, the concentration of the target compounds in surface water (Upper Mekong River) was mostly detected in the rainy season, probably due to the transport of these micropollutants released by wastewater plants and surface runoff from the surrounding area. Eight molecules were detected, and the highest concentration was ketoprofen with an average concentration of 240 ± 6.7 ng.L^{-1}. The concerning result was the presence of caffeine in drinking water.

The proportion of target molecules that were found at a concentration above the LOQ in at least one sample was 66.7% in the Couesnon River water sample, 53.3% in the Upper Mekong River water sample, 60.0% in the effluent WWTP water sample, and 6.7% in drinking water. In surface water, 13 and 11 out of the 15 target micropollutants were determined at least once in the Couesnon River (France) and Upper Mekong River (Cambodia), respectively. The concentrations ranged from 6–975.4 ng.L^{-1} for Couesnon River. The substance with the highest concentration was tramadol. The concentrations for the Upper Mekong River ranged from 5–240 ng;L^{-1}, and the substance with the highest concentration was ketoprofen. Nine substances were widely found in treated effluent from the WWTP, and the substance with the highest concentration was caffeine.

In summary, the developed method was found to be more accurate and perform faster analysis compared to the previous method (Khan et al., 2012) used to analyze the same number of compounds. Sample preparation with the proposed method is simple. The findings provide valuable data to the water treatment plant authorities and can inform policymakers. The finding can represent an alternative to the existing technology used in water treatment plants and improve the overall quality of drinking water in France and Cambodia. The results can help in identifying which substances should be taken into account when assessing water status in the future. It can also help alleviate public health concerns, especially for Cambodia's water.

Conclusions

This study has successfully developed a sensitive and rapid analytical method based on online solid-phase extraction coupled with UPLC-MS/MS runs. This method permits the accurate and rapid multi-residual determination of 15 emerging micropollutants in water at low detection and quantification limits, on average 10 ng.L^{-1} and 30 ng.L^{-1}, respectively, within a total analytical run of seven minutes, including the equilibrium step. The standard addition method was chosen to quantify the compounds in the water sample to overcome the matrix effects and accurately determine the concentrations. Among the studied compounds, diclofenac, diuron, carbamazepine, metazachlor, alachlor, and metolachlor did not experience any significant matrix effect. The applicability of this advanced method was illustrated by analyzing the various samples of treated wastewater, surface water, surface water after coagulation treatment, and drinking water. Many of the target analytes were previously detected at French sample sites, whereas no data record was found for the Cambodian sampling sites. In surface water, 13 and 11 out of 15 of the target micropollutants were detected at least once in the Couesnon River (France) and Upper Mekong River (Cambodia), respectively. The concentrations in the Couesnon River ranged from 6 to 975.5 ng.L^{-1}, with tramadol being the substance with the highest concentration, whereas, in the Upper Mekong River, the concentrations ranged from 5 to 240 ng.L^{-1}, with ketoprofen having the highest concentration. Caffeine was found in the highest concentration in the treated effluent from the Cambodian WWTP, contaminated by 60% of the searched compounds. In conclusion, the developed multi-residual approach will save plenty of time and cost compared to chemical class-directed analysis and reduce the overall workload owing to sample processing. The findings provide valuable data to the water treatment plant authorities and can inform policymakers.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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