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Rapid quantification of pharmaceuticals and pesticides in passive samplers using ultra high performance liquid chromatography coupled to high resolution mass spectrometry

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ABSTRACT

The presence of both pharmaceuticals and pesticides in the aquatic environment has become a well-known environmental issue during the last decade. An increasing demand however still exists for sensitive and reliable monitoring tools for these rather polar contaminants in the marine environment. In recent years, the great potential of passive samplers or equilibrium based sampling techniques for evaluation of the fate of these contaminants has been shown in literature. Therefore, we developed a new analytical method for the quantification of a high number of pharmaceuticals and pesticides in passive sampling devices. The analytical procedure consisted of extraction using 1:1 methanol/acetonitrile followed by detection with ultra-high performance liquid chromatography coupled to high resolution and high mass accuracy Orbitrap mass spectrometry. Validation of the analytical method resulted in limits of quantification and recoveries ranging between 0.2 and 20 ng per sampler sheet and between 87.9 and 105.2%, respectively. Determination of the sampler-water partition coefficients of all compounds demonstrated that several pharmaceuticals and most pesticides exert a high affinity for the polydimethylsiloxane passive samplers. Finally, the developed analytical methods were used to measure the time-weighted average (TWA) concentrations of the targeted pollutants in passive samplers, deployed at eight stations in the Belgian coastal zone. Propranolol, carbamazepine and seven pesticides were found to be very abundant in the passive samplers. These obtained long-term and large-scale TWA concentrations will contribute in assessing the environmental and human health risk of these emerging pollutants.

1. Introduction

Large amounts of various polar anthropogenic pollutants, including pesticides and pharmaceuticals, are continuously introduced into the aquatic environment [1]. As a result, the presence of pharmaceuticals and pesticides in marine waters, typically in the lower ng L^{-1} concentration range, has been reported frequently [2–4]. However, monitoring these pollutants in the marine environment remains an ongoing challenge within the domain of environmental chemistry [5]. Besides the determination of the concentration of these pollutants, modern monitoring techniques should also enable the evaluation of their ecotoxicological effects and the assessment of their environmental and human health risks [6]. Therefore, the determination of time-weighted average (TWA) concentrations over extended sampling periods of these pollutants in the aquatic environment has been put forward.

The most conventional screening technique involves active sampling, which is based on the collection of discrete grab or spot samples of water, and is used in most aquatic monitoring programmes [7]. To obtain long-term and large-scale TWA concentrations, a large number of samples have to be taken, which makes it an expensive and impractical technique [6,7]. Since pharmaceuticals and pesticides are mostly present at trace levels in the marine environment [2–4], large volumes of water need to be collected as well. Although these conventional sampling techniques are very useful, generally, they will not provide appropriate information for assessing the prevalence of pollutants in the marine environment on a long-term basis [8]. As a result, passive

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sampling techniques, which rely on the free flow of pollutants from the sampled medium to a receiving phase namely the sampling device [9], have gained in popularity since most of the disadvantages of active sampling are avoided by this approach [6]. Additionally, passive samplers enable the discrimination between the relevant bioavailable fractions of pollutants from the total amounts present in environmental compartments [10]. In this way, passive sampling or equilibrium-based techniques mimic biological uptake in a more straightforward manner by determining the pollution level of contaminants with respect to their freely dissolved concentration [11,12]. Furthermore, passive samplers are designed to obtain TWA concentrations, providing a more complete picture of organism exposure than those concentrations measured in grab samples, certainly in cases where chemicals bioconcentrate and their environmental concentrations vary temporally [10].

With respect to pharmaceuticals and pesticides, the use of passive sampling devices such as polar organic chemical integrative samplers (POCISs) and Chemcatcher® passive samplers have been recently reported in literature [5,8,13–15]. However, the applicability of these passive sampling devices to characterize the tendency of pharmaceuticals and pesticides to bioaccumulate is limited. In addition, the quantitative aspect is still a major issue of concern, due to both the lack of calibration data to enable quantification of target analytes, as well as the missing insights in the effects of environmental conditions on the analyte uptake [14,16]. In this context, polydimethylsiloxane (PDMS) was preferred as passive sampling material for pharmaceuticals and pesticides in the marine environment in this study. So far, PDMS samplers have mainly been used for quantification of a variety of mostly hydrophobic pollutants [17]. However, Magner et al. [12] demonstrated that PDMS is suitable for mimicking biological uptake of more hydrophilic organic pollutants as well.

Detection of pharmaceuticals and pesticides in complex environmental matrices has generally been performed using liquid chromatography coupled to mass spectrometry (LC–MS/MS) [18,19]. Nevertheless, their analysis at trace concentration levels in aqueous environmental samples remains an important challenge [20]. Nowadays, advances in instrumentation have resulted in a significant progress in the detection of these pollutants in environmental matrices. At first, the use of ultra-high performance liquid chromatography (U-HPLC) enables fast separation of compounds in comparison to conventional LC, due to the use of columns with very small particles [21]. Secondly, with respect to the mass spectrometric detection, accurate mass full scan analysis, using time-of-flight (ToF) and Orbitrap-based mass spectrometers (MS), proved to be a very suitable alternative to triple quadrupole instruments. Full scan data originating from ToF and Orbitrap instruments enable the accurate mass screening of a virtually unlimited number of analytes, targeted as well as untargeted compounds. Typically, the working resolution of an Orbitrap MS amounts up to 100,000 at m/z 200, which is significantly higher than the resolution of a ToF-MS [22]. This high resolving power of Orbitrap MS technology provides higher mass accuracy (<2 ppm) as compared to ToF-MS instruments (<5 ppm) [23]. Especially this high mass resolution and accuracy makes the Orbitrap MS very appropriate for the successful identification of pollutants in environmental samples containing high amounts of matrix co-extracts.

In general, the use of passive sampling devices for quantification of polar micropollutants in marine environments seems promising, however, the applicability for a wide range of compounds remains to be tested [8]. Therefore, we developed a new extraction procedure and analytical method for the quantification of the most frequently used pharmaceuticals in Belgium [4] and the most intensively applied pesticides in Belgium in PDMS passive sampling devices [24]. The method consisted of a liquid extraction using 1:1 methanol/acetonitrile followed by U-HPLC coupled to Orbitrap mass spectrometry (MS). An extensive validation study was carried out to demonstrate the applicability of this analytical approach. In addition, the sampler-water partition coefficients ($K_{sa/wa}$) of the target analytes were determined, to enable quantification of the compounds in the passive samplers. Finally, the optimized method was applied to passive samplers, deployed at several locations in the Belgian coastal zone, to study the presence of pharmaceuticals and pesticides in the Belgian marine environment.

2. Materials and methods

2.1. Study area and sampling

The sampler holders were deployed at eight sampling locations in the Belgian coastal zone: the marinas of Nieuwpoort (NP2), Oostende (OO2), and Zeebrugge (ZB2), the inner side of the harbour of Nieuwpoort (NP1), the outport of Zeebrugge (ZB1), and the location halfway the harbour of Oostende (OO3) were sampled. An additional location was selected at the Sluice Dock in Oostende (OO1) since at this location aquacultural activities take place. Finally, one location was situated in open sea at the Nieuwpoortbank (SEA) (Fig. 1). The samplers were deployed at 1.5–2 m below surface for circa two months from May till July 2008, from March till May 2009 and from mid-July to mid-September 2010. The sampler holders were lost at the SEA-station in 2008 and 2010, at OO1 in 2009, and at OO2 in 2010.

2.2. Reagents and chemicals

The analytical method for pharmaceutical analysis included 16 substances. Paracetamol (99%), ketoprofen (99%), carbamazepine (>99%), diclofenac (>99%), salicylic acid (>99%), clofibric acid (97%), atenolol (≥98%), trimethoprim (≥98%), bezafibrate (≥98%), sulfamethoxazole (99%), pravastatin (≥98%), salbutamol (99%), carprofen (>99%) and chloramphenicol (≥99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ofloxacin (>99%) was obtained from ICN Biomedicals Inc. (OH, USA), while propranolol (>99%) was purchased from Eurogenerics (Brussel, Belgium). The ^{13}C-labeled sulfamethoxazole-phenyl-$^{13}C_6$ (>99%) from Sigma–Aldrich (St. Louis, MO, USA), two deuterated pharmaceuticals, bezafibrate-d_6 (>99%) from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and salicylic acid-d_4 (≥98%) from Toronto Research Chemicals Inc. (North York, ON, Canada), were used as internal standards.

Thirteen pesticides were included in the study. Dichlorvos (>98%), dimethoate (>99%), pirimicarb (≥99%), linuron (>99%), metolachlor (≥98%), chloridazon (≥99%), simazine (>99%), isoproturon (>99%), terbutylazine (>98%), 2,4-D (or 2,4-dichlorophenoxy acetic acid) (>99%) and diuron (>99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA), while atrazine (>99%) and kepone (≥98%) were purchased from Chem Service (West Hester, PA, USA). Isoproturon-d_6 (>99%) and atrazine-d_5 (>99%) from Sigma–Aldrich (St. Louis, MO, USA) were used as internal standards.

Analytical grade solvents were used for extraction and purification purposes, and Optima® LC–MS grade for U-HPLC–MS analysis. They were obtained from VWR International (Merck, Darmstadt, Germany) and Fisher Scientific UK (Loughborough, UK), respectively. Aqueous formic acid (Merck, Darmstadt, Germany) and acetonitrile with formic acid (both 0.08%) were prepared by appropriate dilution of formic acid in ultra-pure water (Arium 611 UV system, Sartorius Stedim Biotech, Aubagne, France) and acetonitrile, respectively.

Primary stock solutions of the pharmaceuticals and pesticides were prepared in ethanol at a concentration of 1 μg μL^{-1}. Working standard mixture solutions were prepared by appropriate dilution

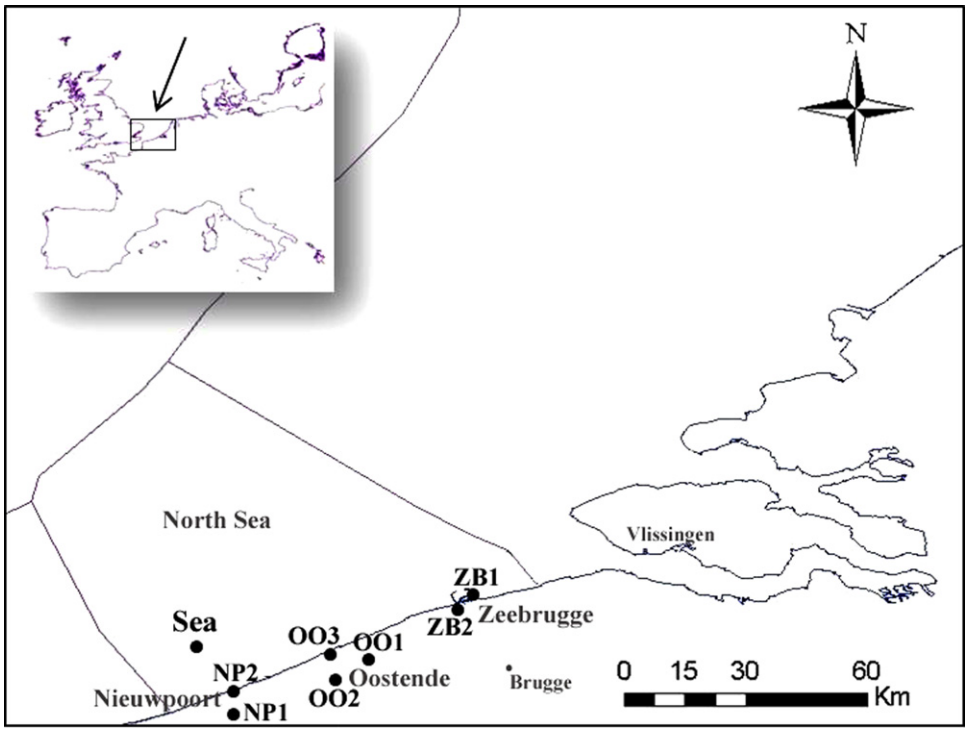

Fig. 1. Study area of the passive sampler experiments in the Belgian coastal zone.

of the stock solutions in ethanol. When necessary, sonication was applied to ensure the complete dissolution of the substances. All solutions were stored at −20 °C in the dark.

2.3. Sampler preparation

The polydimethylsiloxane (PDMS) samplers (AlteSil Laboratory Sheet, Altec Products Ltd., Bude, United Kingdom) with a thickness of 0.5 mm, were cut into sheets of 55 mm × 90 mm, to obtain a total sampling surface of approximately 100 cm^2 and a mean mass of 3.15 g. These sampler sheets were pre-cleaned for 2 h in methanol prior to use. Sampler holders made of stainless steel for mounting the passive samplers were built. The sampler sheets were fixed in such a way that they could move freely, as proposed by Smedes [25]. By this approach, the design does not limit the uptake of the target compounds. After the sampling period, the loaded sampler holders were carefully dismantled and the sheets were transferred on ice to the laboratory where they were stored in a freezer at −20 °C before analysis.

2.4. Extraction and clean-up

As proposed by Rusina [11], the surface of the sampler was cleaned with ultrapure water and wiped dry with a paper tissue before extraction. The internal standards were spiked on the surface of the samplers prior to extraction to a final concentration of 25 ng per sheet. Extraction of a sampler sheet was carried out by adding 20 mL of 1:1 acetonitrile/methanol to a 50 mL tube containing the sheet, followed by shaking this for 60 min and sonication for 60 min. The eluate was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 50 μL methanol and 150 μL of 0.08% aqueous formic acid.

2.5. Chromatography

For both the pesticides and pharmaceuticals, chromatographic separation was carried out using ultra-high performance liquid chromatography (U-HPLC). This U-HPLC-system consisted of an Accela™ high speed LC and an Accela™ autosampler and degasser (Thermo Scientific, San Jose, CA, USA). Chromatographic separation was achieved using a Nucleodur C18 Pyramid U-HPLC column (1.8 μm, 100 mm × 2 mm, Macherey-Nagel, Düren, Germany). For the pharmaceuticals, the mobile phase consisted of 0.08% aqueous formic acid (A) and 0.08% formic acid in acetonitrile (B). A linear gradient was used, starting from 98% A to 2% B, which was held for 0.8 min. In 30 s the percentage of acetonitrile was increased to 65% B, which was held for 0.7 min. The percentage of acetonitrile was increased further to 100% B in 1 min and held for 2 min. Equilibration at initial conditions was done for 2.5 min. Pesticide separation was achieved using 0.08% aqueous formic acid (A) and methanol (C). The linear gradient started with a mixture of 98% A and 2% C for 1 min. The methanol percentage increased to 90% in 30 s, and further to 100% in 3 min, which was held for 1 min. Between samples, the column was allowed to equilibrate at initial conditions for 1 min. The injection volume was 10 μL. The column oven and tray temperature were 25 °C and 15 °C, respectively.

2.6. Mass spectrometric detection

Detection of pharmaceuticals and pesticides was carried out using an Exactive™ Benchtop Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated electrospray ionization probe (HESI-II). The Exactive™ is an Orbitrap-based MS, which was operated alternating from positive to negative ion mode, with both scan types at a resolution of 50,000 at 2 Hz (2 scans per second). For the compounds of interest, a scan range of m/z 80–800 was chosen. The automatic gain control (AGC) target was set at ultimate mate accuracy (5×10^5) and the maximum injection time was 500 ms. The instrumental settings were optimized to maximize the signal. The parameters as presented in Table 1 were found to be the optimal ionization source working parameters for the respective analytes. Initial instrument calibration was done by infusing calibration mixtures for positive and negative ion mode

Table 1
HESI-II working parameters for ionization of the selected pharmaceuticals and pesticides.

	Pharmaceuticals	Pesticides
Spray voltage (kV)	4.0	4.0
Sheath gas flow rate (arbitrary units, au)	30	30
Auxiliary gas flow rate (au)	0	0
Capillary temperature (°C)	275	250
Heater temperature (°C)	250	350
Capillary voltage	82.5 (−30.0)	82.5 (−30.0)
Tube lens voltage	170.0 (−95.0)	120.0 (−95.0)
Skimmer voltage	20.0 (−26.0)	20.0 (−26.0)

(Thermo Fisher Scientific, San Jose, CA, USA). The positive calibration mixture included caffeine, MRFA and Ultramark® 1621, while the negative calibration solution comprised sodium dodecyl sulfate, sodium taurocholate and Ultramark® 1621. These compounds were dissolved in a mixture of acetonitrile, water and methanol, and both mixtures were infused using a Chemyx Fusion 100 syringe pump (Thermo Fisher Scientific, San Jose, CA, USA). The option of "all-ion fragmentation" using the High Energy Collision Dissociation (HCD) cell was turned off. The forevacuum, high vacuum and ultra high vacuum were maintained around 2 mbar, from $1E^{-05}$ to $3E^{-05}$, and below $8E^{-10}$ mbar, respectively. Instrument control and data processing were carried out by means of Xcalibur 2.1 and ToxID software (Thermo Electron, San Jose, CA, USA).

2.7. Determination of $K_{sa/wa}$

This experimental setup was based on the study of Magner et al. [12]. Thirty-three sheets were placed in a beaker filled with 5 L of ultra-pure water under gentle stirring at 100 rpm. The water was spiked with all pharmaceuticals at a concentration of 20 $\mu g\,L^{-1}$, except propranolol and carbamazepine, which were spiked at 5 $\mu g\,L^{-1}$. The pesticides were spiked at 5 $\mu g\,L^{-1}$, apart from dichlorvos, 2,4-D, linuron and kepon, which were spiked at 20 $\mu g\,L^{-1}$. Three 1 mL water samples and three sheets were sampled after 0, 0.08, 0.75, 1, 2, 3, 5, 6, 7, 8, 9 d, respectively. After sampling, the sheets were left to dry on a paper towel and analyzed as described above. The water samples were analyzed by direct injection of 10 μL into the U-HPLC Orbitrap MS instrument, using the optimized analytical parameters. The $K_{sa/wa}$ ($L\,kg^{-1}$) is defined as: $K_{sa/wa} = C_{sa}/C_{wa}$ with C_{sa} ($g\,kg^{-1}$) and C_{wa} ($g\,L^{-1}$) as the concentrations of the analyte in the sampler and the water phase, respectively. Knowledge of the $K_{sa/wa}$ of the analytes is required to enable quantification of the compounds in the equilibrium based passive samplers. Each time water and sheets were sampled, the mean ($n = 3$) water and sampler concentrations of the analytes were calculated. Equilibrium between the sampler and the water phase was achieved when the concentration of the analyte in the water phase remained constant throughout the experiment.

2.8. Identification and quantification

The target analytes were identified based on both their retention time relative to that of the internal standards, and their accurate mass. According to previous studies using Orbitrap MS, a maximum mass deviation of 5 ppm was allowed within this study [20,22,26].

So far, appropriate identification criteria using these modern instruments based on high-resolution accurate mass spectrometry are incomplete in the commonly used procedure prescribed by Commission Decision 2002/657/EC [20,27]. Both the criteria concerning mass resolution and mass accuracy, as well as the system of identification points have not been fully specified for these MS systems yet. Therefore, as was suggested by several authors [21,23,28], additional criteria for the use of these accurate mass LC–MS technologies should be implemented in the standardized validation procedures. Nevertheless, using maximum mass deviations of 5 ppm, a high reliability in identification can be expected.

Upon identification, area ratios were determined by integration of the area of an analyte within the obtained chromatograms in reference to the integrated area of the internal standard. The analyte concentrations were calculated by fitting their area ratios to a ten-point calibration curve in the sample matrix. To this end, sheets were spiked with a standard mixture obtaining ten final concentrations in the range of 0.01–100 ng per sheet and with a final concentration of 25 ng per sheet of the internal standards.

2.9. Quality assurance

Before and after analysis of a series of samples, a standard mixture (0.1 ng on column) of the targeted analytes and the internal standards was injected to check the performance of the U-HPLC Orbitrap MS system. Quality control of the method was performed by analysis of a blank sample, together with linear calibration curves constructed using matrix samples spiked with standard solutions at ten concentration levels ranging from 0.01 to 100 ng per sheet. This was performed for every series of samples at least in duplicate.

3. Results and discussion

3.1. Extraction of the samplers: recovery optimization

3.1.1. Spiking of the samplers

Spiking of passive samplers is generally performed according to Booij et al. [29]. This method is based on equilibration of the samplers in aqueous/methanolic solutions of the compounds. However, this method failed for most pharmaceuticals and pesticides within this study, with low uptake percentages for most compounds. This can be attributed to the low sampler to water and methanol partition coefficients of polar compounds. Therefore, an alternative method was employed by directly spiking the target compounds and internal standards onto the PDMS sampler surface, and allowing the solvent carrier to volatilize [30]. This spiking method resulted in high uptake percentages of all compounds and was further applied within this study.

3.1.2. Optimization of the extraction procedure

Generally, methanol has been reported to be the appropriate extraction solvent for compounds with $\log K_{ow} < 8$, with acetonitrile as a very good alternative [11]. Recently, a 3:1 mixture of acetonitrile/water with 1% formic acid and a 1:1 mixture of methanol/acetonitrile provided the best results in extracting the same pharmaceuticals and pesticides from biotic tissue, respectively [31]. Therefore, both mixtures as well as the separate solvents methanol and acetonitrile were tested as extraction solvents for the targeted compounds. In addition, different extraction volumes (20 vs. 40 mL) and extraction conditions (sampler sonication, sampler shaking or both) were tested. Therefore, sheets were spiked with the targeted compounds at three concentration levels (10, 50 and 100 ng per sheet) and mean extraction efficiencies of each analyte were calculated upon U-HPLC–MS analysis. The optimal extraction parameters were determined based on both the extraction efficiencies and the clarity of the extract. The best results were obtained by adding 20 mL of the 1:1 mixture of methanol and acetonitrile to a PDMS sheet, and allowing this to shake and sonicate, both for 60 min. The extraction efficiencies of the pharmaceuticals and pesticides ranged, respectively, between 49 and 99% and between 42 and 92%.

3.2. Chromatography and mass spectrometry detection

3.2.1. Chromatography

Recently, the development and optimization of new U-HPLC methods for rapid chromatographic separation of pharmaceuticals and pesticides for analysis of marine organisms was reported by Wille et al. [31]. The same methods were set up in front of the Orbitrap MS, allowing good separation of the targeted compounds for our application. The chromatograms obtained upon analysis of a PDMS sheet spiked at ten times the LOQ level are shown in Figs. 2 and 3, respectively.

3.2.2. Orbitrap MS

The excellent applicability of Orbitrap MS for metabolomic and proteomic applications has been demonstrated in literature [22,32,33]. The suitability of Orbitrap MS for the identification of a large number of pharmaceuticals in aqueous matrices, has been demonstrated as well [20]. To the best of our knowledge, the use of Orbitrap MS for the quantification of pesticides and pharmaceuticals in environmental matrices, including passive samplers, has not been reported earlier.

First, the observed masses were compared with the theoretical masses by direct infusion of individual analytes (10 ng μL^{-1}) into the heated electrospray ionization source (HESI-II). Next, the ionization source working parameters for the targeted analytes were one after the other optimized by analyzing a standard mixture (0.1 ng μL^{-1}). The optimal values of these parameters were determined based on the peak intensities, areas, S/N ratios and peak shape of the individual analytes. Since the tube lens voltage depends on the molecular structure, different values were obtained for the pharmaceuticals and pesticides in positive ion mode: 170 V and 120 V, respectively. Different temperatures for both groups were found as well (Table 1).

Before the ions are injected into the Orbitrap, they are trapped in a curved RF-only quadrupole, the C-trap. To avoid space charging [22,34], the number of ions present in the C-trap is controlled by the use of the Automatic Gain Control (AGC). The AGC target determines the number of charges collected for every scan. Three AGC values are possible: 3×10^6 for a high dynamic range scan, 1×10^6 for a balanced scan and 5×10^5 for ultimate mass accuracy. Standard mixtures of the analytes (0.1 ng μL^{-1}) were analyzed using these three possible AGC values. Based on peak shape and width, area, signal to noise ratio and mass deviation, the optimal AGC target value was found to be 5×10^5 ions. The ion density in the C-trap was kept as low as possible to ensure the best resolution and mass accuracy, without a significant loss of sensitivity. Besides the AGC target, another crucial parameter using the Orbitrap MS is the mass resolution. In recent years, several studies have reported the effect of the resolving power on analytical results [22,26]. Standard mixtures of the analytes (0.1 ng μL^{-1}) were analyzed using mass resolution values varying between 10,000 and 100,000. A resolution of 50,000 at 2 Hz (2 scans per second) proved to be the best compromise between peak shape and width, mass deviation and datapoints over the chromatographic peak for this application. Therefore, a resolution of 50,000 was further applied within this study.

Identification of compounds was, together with the retention time, based on their accurate mass, i.e. by matching the theoretical mass with the observed mass. Therefore, the expected or theoretical masses of the target compounds were calculated to four decimal places, using the Xcalibur software (Tables 2 and 3). The mass accuracy or mass deviation was expressed in parts per million (ppm) and was defined as: $10^6 \times$ [(measured mass − theoretical mass)/theoretical mass]. Extracted ion chromatograms (EIC) were obtained using a 5 ppm window. The mean mass deviations of all the compounds were calculated at LOQ level ($n = 10$) and were presented in Tables 2 and 3, as well as the ion mode and retention times. The mass deviations obtained were below 2 ppm for most analytes, indicating a high mass accuracy. Propranolol, isoproturon and atrazine showed slightly higher mass deviations, while for salicylic acid a mass deviation of 4.8 was obtained. The relatively higher mass deviation of salicylic acid, the only compound with m/z ratio below 150, can be attributed to the presence of many background ions in the lower mass area [35]. The same experiences were reported in literature: mass deviations between 1 and 3 ppm for compounds with m/z ratio higher than 150, while a 5 ppm error was observed for compounds with $m/z < 150$ [20].

3.3. Method validation

The newly developed analytical method was validated according to the criteria specified in CD 2002/657/EC [27] for quantitative confirmation as well as to the guidelines of SANCO/10684/2009 [36] on pesticide residues analysis in food and feed. In practice, validation of the method was executed by adopting the protocol proposed by Antignac et al. [37]. This protocol was tailored for validating analytical methods based on MS detection and offers a compromise between CD 2002/657/EC [27] and practical aspects and limitations related to laboratory work.

The use of isotopically labeled internal standards in MS-based chemical analysis has been highly recommended [27,38]. For the pharmaceuticals, one ^{13}C-labeled sulfamethoxazole-phenyl-$^{13}C_6$ and two deuterated pharmaceuticals, bezafibrate-d_6 and salicylic acid-d_4, were used as internal standards. Isoproturon-d_6 and atrazine-d_5 were selected as the internal standards for the pesticides. The corresponding internal standards were used for sulfamethoxazole, bezafibrate, salicylic acid, isoproturon and atrazine, while the most appropriate internal standard available was used for the other compounds (Tables 2 and 3). These internal standards were supplemented to every sampler prior to extraction to a final concentration of 25 ng per sheet. The results obtained were thus corrected for possible matrix-induced suppression or enhancement effects.

3.3.1. Specificity

The specificity of the methods was demonstrated by analysis of blank sampler sheets ($n = 6$) and sheets fortified with each analyte separate at their LOQ level. Sheets spiked with a mixture of all analytes at LOQ level were analyzed as well. None of the compounds were detected in the blanks. The obtained chromatograms showed a significant increase in peak area and intensity at the specific retention time of the compounds. The specificity of these analytical approaches were confirmed since no other significant peaks with a signal-to-noise ratio of 3 or more were observed at the specific retention times of the targeted pharmaceuticals and pesticides (Figs. 2 and 3). Using Orbitrap MS, the specificity is guaranteed by the high resolving power of the instrument [22].

3.3.2. Selectivity

Analytes were identified on the basis of their relative retention time, which is the ratio of the retention time of the analyte to that of the internal standard. In addition, the accurate mass of the ions ($[M-H]^-$ or $[M-H]^+$) in the spectrum was taken into account when the chromatographic peak of interest had a signal-to-noise ratio of at least 3:1. A maximum mass deviation of 5 ppm was allowed within this study.

3.3.3. Linearity

The linearity of the developed methods was evaluated for each target compound by preparing ten-point calibration curves

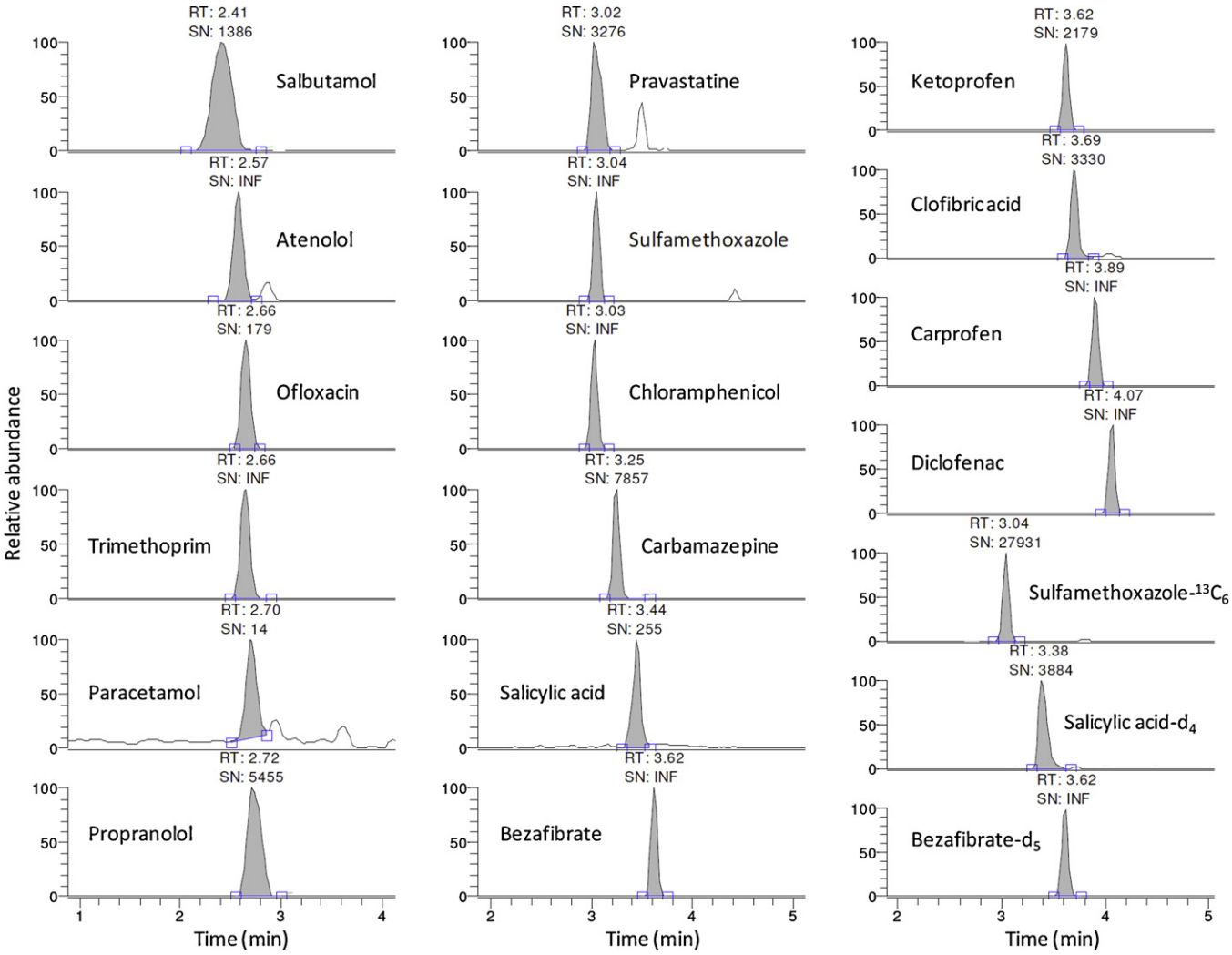

Fig. 2. Chromatograms of a passive sampler sheet fortified with the target pharmaceuticals at ten times the LOQ level.

(3 replicates). Blank sheets were spiked with a standard mixture obtaining concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 25, 50 and 100 ng per sampler sheet of the targeted pharmaceuticals and pesticides. Linear regression analysis was executed by plotting the peak area ratios of the analyte against the internal standard versus the analyte concentration. The mean correlation coefficients of the calibration curves were >0.99, indicating good linearity in this concentration range (Tables 4 and 5). Only dichlorvos, for which R^2 equaled 0.97, showed slightly inferior linearity.

Table 2
Ion mode, internal standard used, retention time, accurate mass and mean mass deviation of the targeted pharmaceuticals.

Compound	Ion mode	Internal standard used	tR (min)	Accurate mass (*m*/*z*)	Mean mass error (ppm)
Salbutamol	+	Salicylic acid-d_4	2.41	240.1594	1.10
Atenolol	+	Sulfamethoxazole-$^{13}C_6$	2.57	267.1703	1.37
Ofloxacin	+	Sulfamethoxazole-$^{13}C_6$	2.66	362.1511	1.58
Trimethoprim	+	Sulfamethoxazole-$^{13}C_6$	2.66	291.1452	0.95
Paracetamol	+	Salicylic acid-d_4	2.70	152.0706	0.50
Propranolol	+	Sulfamethoxazole-$^{13}C_6$	2.72	260.1645	2.27
Pravastatin	+	Salicylic acid-d_4	3.02	447.2357	1.27
Sulfamethoxazole	+	Sulfamethoxazole-$^{13}C_6$	3.03	254.0594	0.77
Chloramphenicol	–	Sulfamethoxazole-$^{13}C_6$	3.03	321.0051	0.93
Carbamazepine	+	Salicylic acid-d_4	3.25	237.1022	0.72
Salicylic acid	–	Salicylic acid-d_4	3.44	137.0244	4.80
Bezafibrate	+	Bezafibrate-d_6	3.62	362.1154	0.99
Ketoprofen	+	Salicylic acid-d_4	3.62	255.1016	1.47
Clofibric acid	–	Bezafibrate-d_6	3.69	213.0324	0.95
Carprofen	–	Salicylic acid-d_4	3.89	272.0484	1.69
Diclofenac	+	Salicylic acid-d_4	4.07	296.0240	1.75
Sulfamethoxazole-$^{13}C_6$	+		3.04	260.0795	0.31
Salicylic acid-d_4	–		3.38	141.0495	4.51
Bezafibrate-d_6	+		3.62	368.1530	1.06

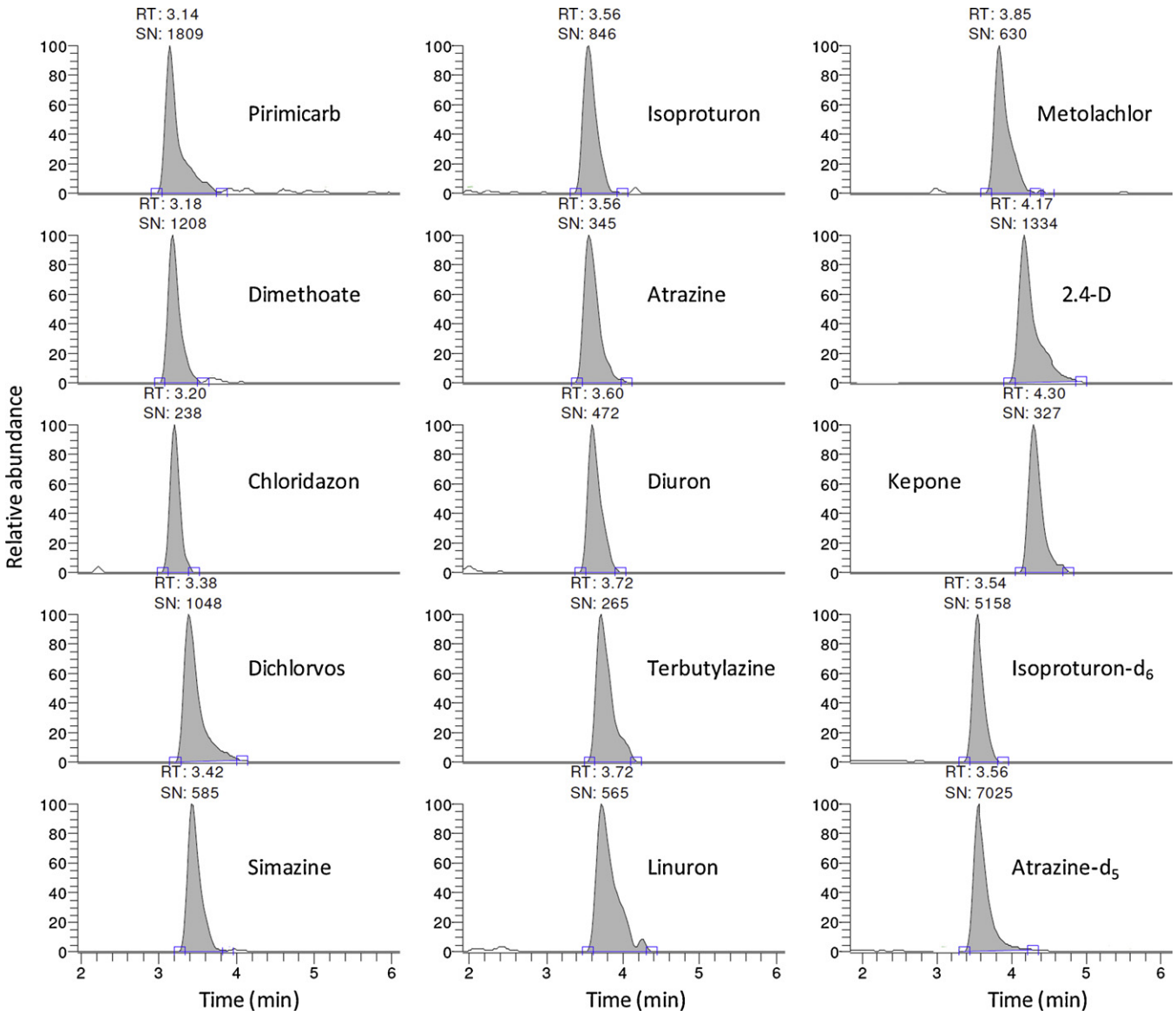

Fig. 3. Chromatograms of a passive sampler sheet fortified with the target pesticides at ten times the LOQ level.

3.3.4. *Limit of detection and quantification*

Limits of detection (LODs) and quantification (LOQs) were determined based on the outcome of the ten-point calibration curves of the previous section. The concentrations of the analytes were calculated using the overall equation of the calibration curves. The LOD was defined as the lowest detectable concentration of the calibration curve with a signal-to-noise ratio of at least 3:1. The LOQs were then determined as the final LOD multiplied by 2 [39].

Table 3
Ion mode, internal standard used, retention time, accurate mass and mean mass deviation of the targeted pesticides.

Compound	Ion mode	Internal standard used	tR (min)	Accurate mass (*m*/*z*)	Mean mass error (ppm)
Pirimicarb	+	Isoproturon-d_6	3.14	239.1503	0.55
Dimethoate	+	Isoproturon-d_6	3.18	230.0069	1.50
Chloridazon	+	Atrazine-d_5	3.20	222.0429	1.50
Dichlorvos	+	Atrazine-d_5	3.38	220.9532	1.83
Simazine	+	Isoproturon-d_6	3.42	202.0854	1.77
Isoproturon	+	Isoproturon-d_6	3.56	207.1492	2.14
Atrazine	+	Atrazine-d_5	3.56	216.1010	2.30
Diuron	+	Isoproturon-d_6	3.60	233.0243	1.42
Terbutylazine	+	Atrazine-d_5	3.72	230.1167	1.93
Linuron	+	Isoproturon-d_6	3.72	249.0192	1.78
Metolachlor	+	Atrazine-d_5	3.85	284.1412	1.82
2,4-D	–	Atrazine-d_5	4.16	218.9621	1.53
Kepone	–	Isoproturon-d_6	4.30	506.68260	0.76
Isoproturon-d_6	+		3.54	213.1869	1.37
Atrazine-d_5	+		3.56	221.1324	1.79

Table 4
Validation parameters, log K_{ow} and $K_{sa/wa}$ values of the targeted pharmaceuticals.

Compound	Extraction efficiency (%)	LOD (ng sheet^{-1})	LOQ (ng sheet^{-1})	R^2	Recovery (mean ± SD%)	Repeatability (RSD%)	Within-lab Reproducibility (RSD%)	Log K_{ow}	$K_{sa/wa}$ (L kg^{-1})	Log $K_{sa/wa}$ (L kg^{-1})	Log BAF (L kg^{-1})
Salbutamol	97	5	10	>0.99	101.6 ± 8	17.9	17.5	0.64	0.04	−1.42	–
Atenolol	80	0.5	1	>0.99	99.1 ± 11	17.5	18.2	0.16	0.04	−1.38	–
Ofloxacin	65	0.5	1	>0.99	89.4 ± 9	14.6	14.7	−0.39	0.21	−0.69	–
Trimethoprim	58	0.5	1	>0.99	92.6 ± 11	16.9	18.0	0.91	0.68	−0.17	–
Paracetamol	92	0.5	1	>0.99	98.9 ± 7	15.2	15.5	0.46	0.05	−1.29	–
Propranolol	49	0.5	1	>0.99	94.0 ± 9	16.9	16.5	3.48	23.52	1.37	1.5 ± 0.6 (n = 8)
Pravastatin	82	5	10	>0.99	87.9 ± 10	18.1	19.0	3.10	0.06	−1.23	–
Sulfamethoxazole	82	0.5	1	>0.99	102.1 ± 9	14.7	14.7	0.89	0.09	−1.04	–
Chloramphenicol	72	0.5	1	>0.99	101.6 ± 11	14.5	16.1	1.14	0.09	−1.04	–
Carbamazepine	93	0.5	1	>0.99	100.6 ± 7	10.2	10.6	2.45	30.97	1.48	1.1 ± 0.4 (n = 32)
Salicylic acid	98	0.5	1	>0.99	102.4 ± 15	18.4	19.3	2.26	0.16	−0.79	–
Bezafibrate	75	0.5	1	>0.99	93.0 ± 6	10.8	10.9	4.25	0.04	−1.41	–
Ketoprofen	72	5	10	>0.99	88.0 ± 12	14.9	16.1	3.12	0.29	−0.54	–
Clofibric acid	60	1	2	>0.99	99.3 ± 8	11.2	10.8	2.57	0.08	−1.11	–
Carprofen	75	0.5	1	>0.99	93.2 ± 10	20.2	20.3	3.79	1.81	0.26	–
Diclofenac	99	1	2	>0.99	100.3 ± 9	17.0	19.6	4.51	2.40	0.38	–

The LOQs of the targeted pharmaceuticals and pesticides ranged, respectively, between 1 and 10 ng per sheet and between 0.2 and 20 ng per sheet. These LOQs are considered highly satisfactory, despite the absence of comparable data in literature.

3.3.5. Trueness

Since no certified reference material was available, trueness of the measurements was assessed by analysis of blank sheets spiked with each analyte at LOQ level, two times the LOQ level and ten times the LOQ level (recovery). This was performed in six replicates for all three concentration levels. Mean recoveries of the targeted pharmaceuticals and pesticides (n = 18) varied, respectively, between 87.9 and 102.4% and between 94.2 and 105.2% (Tables 4 and 5). These calculated recoveries fulfill the criteria set by CD 2002/657/EC [27] and SANCO/10684/2009 [36], for which typically a recovery is required within the range of 80–110% and 70–120%, respectively.

3.3.6. Precision

Evaluation of the precision included the determination of the repeatability and the within-laboratory reproducibility of these new methods. Both validation parameters were evaluated by calculating the relative standard deviation (%RSD). To study the repeatability of the method, three series of six replicates of sheets were analyzed, and this at three concentration levels: LOQ level, two times the LOQ level and ten times the LOQ level. These analyses were carried out by the same analyst under repeatable conditions. As presented in Tables 4 and 5, the calculated RSD values for most compounds were below 20%, indicating good repeatability according to SANCO/2007/3131 [36]. Only carprofen showed a slightly inferior repeatability (%RSD of 20.2). The criterion of CD 2002/657/EC [27], demanding RSD values below 15%, was achieved for half of the pharmaceuticals and most pesticides.

For evaluation of the reproducibility only the within-laboratory reproducibility was evaluated. Therefore, four series of six replicates of fortified sheets were analyzed, and this at three concentration levels: LOQ level, two times the LOQ level and ten times the LOQ level. Different analysts carried out these analyses on different days, using different spiking solutions and sampling sheets. According to CD 2002/657/EC [27] and SANCO/2007/3131 [36], typically a reproducibility RSD $\leq$ 20% is required. As can be deduced from Tables 4 and 5, except for carprofen and dichlorvos, all RSD values were satisfactory. The higher RSD value of dichlorvos and carprofen could be attributed to the absence of an appropriate

Table 5
Validation parameters, log K_{ow} and $K_{sa/wa}$ values of the targeted pesticides.

Compound	Extraction efficiency (%)	LOD (ng sheet^{-1})	LOQ (ng sheet^{-1})	R^2	Recovery (mean ± SD%)	Repeatability (RSD%)	Within-lab Reproducibility (RSD%)	Log K_{ow}	$K_{sa/wa}$ (L kg^{-1})	Log $K_{sa/wa}$ (L kg^{-1})	Log BAF (L kg^{-1})
Pirimicarb	42	0.5	1	>0.99	97.9 ± 11	9.5	10.5	1.70	356.4	2.52	–
Dimethoate	49	0.5	1	>0.99	103.3 ± 14	12.7	13.1	0.78	0.45	−0.35	–
Chloridazon	55	1	2	>0.99	103.8 ± 9	17.4	17.5	1.14	0.79	−0.10	–
Dichlorvos	57	10	20	0.97	94.9 ± 9	18.4	25.5	1.43	180.5	2.24	–
Simazine	65	0.5	1	>0.99	104.3 ± 10	15.1	14.1	2.18	112.7	2.05	–
Isoproturon	55	0.1	0.2	>0.99	100.7 ± 7	9.4	9.7	2.87	118.2	2.07	1.1 ± 0.3 (n = 8)
Atrazine	67	0.1	0.2	>0.99	102.5 ± 10	10.6	11.7	2.61	162.0	2.20	–
Diuron	50	0.5	1	>0.99	100.5 ± 12	12.9	14.7	2.68	138.3	2.13	1.1 ± 0.3 (n = 35)
Terbutylazine	62	0.1	0.2	>0.99	99.7 ± 9	14.8	15.1	3.21	332.5	2.40	0.6 ± 0.3 (n = 8)
Linuron	69	5	10	>0.99	96.5 ± 10	11.4	11.3	3.20	–	–	–
Metolachlor	49	0.5	1	>0.99	94.2 ± 9	9.7	10.4	3.13	2534.8	3.40	1.7 ± 0.5 (n = 22)
2,4-D	92	1	2	>0.99	103.1 ± 4	6.7	6.9	2.81	0.1	−1.29	–
Kepone	88	10	20	>0.99	105.2 ± 9	19.0	19.8	5.41	–	–	–

internal standard. The use of an internal standard with a higher structural similarity could result in lower RSD values [40]. However, the commercial availability of labeled internal standards is limited and the criteria for good reproducibility were only slightly exceeded using the most appropriate internal standards.

3.4. Determination of $K_{sa/wa}$

A 9-d experiment was conducted, to determine the sampler-water partition coefficients ($K_{sa/wa}$). The pharmaceuticals propranolol and carbamazepine showed a high affinity for the PDMS samplers, resulting in $K_{sa/wa}$ values of 23.5 and 31.0 L kg^{-1}, respectively. The $K_{sa/wa}$ value obtained for carbamazepine was to a large extent in accordance with the value reported by Magner et al. [12]. For propranolol and carbamazepine, the enrichment profile is depicted in Fig. 4, using the logarithm of $K_{sa/wa}$ as *y*-axis. Carprofen and diclofenac showed a moderate affinity for the PDMS samplers, while for the other pharmaceuticals, the $K_{sa/wa}$ values obtained were <1 L kg^{-1}, indicating a lower affinity for the sampler compared to the water phase. On the other hand, the affinity for the PDMS sampler was demonstrated for most pesticides, reaching equilibrium within 3 d (Fig. 4). Except for chloridazon, dimethoate and 2,4-D, all $K_{sa/wa}$ values were above 1 L kg^{-1}, ranging from 112.7 L kg^{-1} for simazine to 2534.8 L kg^{-1} for metolachlor. The $K_{sa/wa}$ value of atrazine was 162.0 L kg^{-1}, which is in the same order of magnitude as the partition coefficient of 153 L kg^{-1} reported in literature [10]. Using this experimental setup, no $K_{sa/wa}$ values could be established for linuron and kepone. Probably, the $K_{sa/wa}$ values of both pesticides were too high. As a consequence, the initial concentration of these compounds in the water phase was significantly influenced by the samplers. This depletion phenomenon prevented the determination of $K_{sa/wa}$ for these compounds.

The logarithmic function of the octanol–water partition coefficient of each compound ($\log K_{ow}$), generally used as a criterion for hydrophobicity [41], is presented in Tables 4 and 5 as well [42]. Typically, the $\log K_{sa/wa}$ of an analyte is lower than its $\log K_{ow}$, except for pirimicarb, dichlorvos and metolachlor. However, no correlation between both partition coefficients was observed (R^2 of 0.097 and *p*-value of 0.114). Obviously, the sampler-water partitioning is not exclusively driven by the hydrophobicity of the substances, but compound-specific interactions in the sampler phase are important as well [43].

The $K_{sa/wa}$ coefficients were determined on a standardized manner, as described by Magner et al. [12], without making a distinction between different values of pH, salinity and temperature. According to current literature [5,14,16], these environmental parameters may definitely impact the uptake of pollutants into the samplers. Therefore, an intensive separate study dealing with the effects of environmental conditions on the analyte uptake is desired, however, this was outside the scope of the present study. Consequently, further research should concentrate on improved approaches for calibration and quantification of PDMS passive samplers, thereby taking the different environmental parameters into consideration.

3.5. Application to passive sampler samples deployed in the Belgian coastal zone

3.5.1. Targeted compounds

Passive samplers were deployed for circa two months at eight sampling locations in the Belgian coastal zone in 2008, 2009 and 2010. Compounds with $K_{sa/wa}$ < 1 showed greater affinity for the water phase than for the PDMS passive sampler. As a consequence, the reliable calculation of TWA concentrations of these compounds using the samplers was inhibited [44]. Therefore, only the pharmaceuticals and pesticides with $K_{sa/wa}$ > 1 were considered for quantification. First, the analytes were measured in the samplers using the optimized extraction and U-HPLC Orbitrap MS methods as described above. Next, the concentrations of the compounds in the water phase were calculated using the following equation: $C_{wa} = C_{sa}/K_{sa/wa}$, expressed in nanograms per liter. These obtained concentrations may be considered as approximate calculated TWA concentrations, since the possible impact of the environmental conditions was not taken into consideration (see Section 3.4). As can be seen from Table 6, two pharmaceuticals were detected in all samplers: the β-blocker propranolol and the psychiatric drug carbamazepine in concentrations up to 7294 ng L^{-1} and 732 ng L^{-1}, respectively. Propranolol and carbamazepine have been found in grab water samples collected in the same study area, in concentrations up to 24 ng L^{-1} and 321 ng L^{-1} [4]. Obviously, propranolol was quantified in significantly higher concentration levels using the equilibrium based passive samplers in comparison with grab water samples. A possible explanation is the decreasing hydrophilicity and thus higher affinity for the PDMS sampler of propranolol, due to the increasing salinity in the marine environment [5]. The rather high Setschenow salting-out constant of propranolol of 3.29 could significantly affect the $K_{sa/wa}$ value [45]. Assuming a salinity of 30 g L^{-1}, the $K_{sa/wa}$ value will increase with a factor of about 50 [46]. Much more realistic TWA concentrations, in the low ng L^{-1} range, were found if this salting out effect was taken into consideration. Carbamazepine was detected in every passive sampler as well. The calculated water concentrations were within the same order of magnitude with levels detected in grab samples. As a result, the salting-out effect was expected to be low [4,47]. Due to its high persistence, carbamazepine has been reported as an excellent tracer substance for pharmaceutical contamination [14,48]. From these results it may be concluded that the use of PDMS samplers to obtain long-term and large-scale TWA concentrations of carbamazepine, as a representative of the pharmaceuticals, could be very useful in revealing pharmaceutical contamination of the marine environment. By this approach, using the PDMS samplers, both the pollution level of hydrophobic compounds for which they were initially designed (PAHs, PCBs, ...) as well as the more hydrophilic pollutants (pharmaceuticals represented by carbamazepine, pesticides, ...) could be estimated simultaneously.

As shown in Table 6, seven pesticides were very frequently detected in the passive sampler extracts. Calculation of the TWA concentrations resulted in concentration levels of the pesticides up to 118 ng L^{-1} for pirimicarb, 164 ng L^{-1} for metolachlor, 56 ng L^{-1} for atrazine, 263 ng L^{-1} for diuron, 260 ng L^{-1} for isoproturon, 159 ng L^{-1} for simazine and 469 ng L^{-1} for terbutylazine. These values are in line with reported levels of pesticides found in traditional grab samples from the same study area: maximum detected concentrations were 77 ng L^{-1} for atrazine, 454 ng L^{-1} for diuron, 292 ng L^{-1} for isoproturon, 60 ng L^{-1} for simazine and 347 ng L^{-1} for terbutylazine [49]. According to the Water Framework Directive (2000/60/EC) [50] and its daughter directive (2008/105/EC) [51], environmental quality standards (EQSs), expressed as annual average values, were established for atrazine, diuron, isoproturon and simazine being 0.6, 0.2, 0.3 and 1 μg L^{-1}, respectively. The calculated diuron concentrations exceeded these EQSs twice: at sampling locations OO2 and ZB2, both in 2008. The EQSs for the other compounds were however never exceeded. The comparison with EQSs presents only a preliminary approach to characterize the environmental risks to aquatic ecosystems and organisms. To aid in assessing these risks, quantitative–structure–activity relationships (QSARs) [52–54] have recently been developed to generate screening and toxicity data. However, confirmation by direct measurements of concentrations in water is definitely required [10]. Therefore, these obtained concentration via passive samplers measurements are very useful, in particular because TWA concentrations of the relevant bioavailable fraction of the target pharmaceuticals and pesticides are provided.

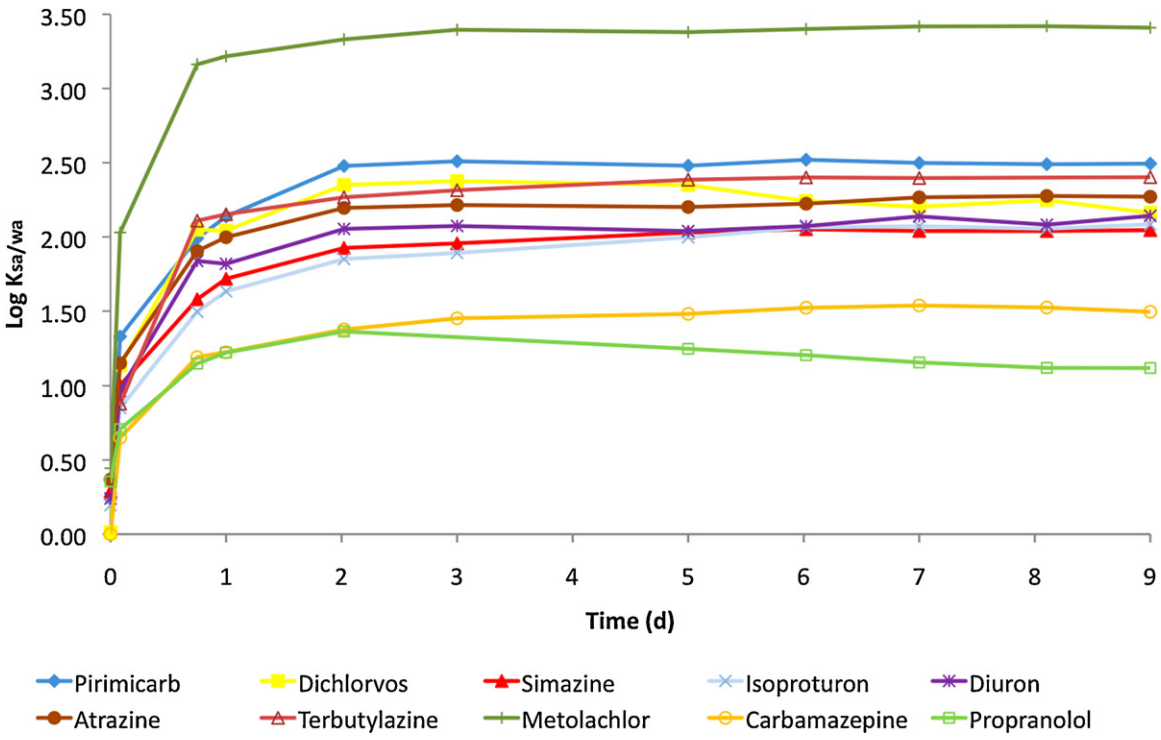

Fig. 4. Enrichment profile of the two pharmaceuticals and eight pesticides with high affinity for the PDMS passive samplers.

Typically, the highest concentrations of the pharmaceuticals and pesticides were found at the sampling points in the harbours; more specific those of Nieuwpoort and to a lesser extent Oostende. Both locations receive major inputs of contaminated surface water, resulting in the increased presence of the targeted pharmaceuticals and pesticides. Due to both dilution and degradation effects, only few target compounds were found at the SEA-station, and this at rather low concentrations in comparison with the harbour stations.

The present study was conducted side-by-side with the recent publication results reported by Wille et al. [31] on the accumulation of the same pharmaceuticals and pesticides in marine organisms. In this way, the commonality in contaminants and concentrations accumulated by these two matrices may be determined. Bioconcentration of several pharmaceuticals in *Mytilus edulis* has been observed, including propranolol and carbamazepine, which were found to be present in the passive samplers as well. Also four pesticides have been found both in tissue and samplers: diuron, isoproturon, terbutyazine and metolachlor. Apparently, a correlation between analyte concentrations in side-by-side exposures of biota and samplers exists for several pharmaceuticals and pesticides. The tendency of an organism to bioaccumulate is assessed by the bioaccumulation factor (BAF) which can be calculated using the following equation [55]:

$$\mathrm{BAF} = \frac{C_{\mathrm{biota}}}{C_{\mathrm{water}}}$$

BAF values are expressed in $\mathrm{L\,kg^{-1}}$, since the biota concentration (C_{biota}) is expressed in $\mu\mathrm{g\,kg^{-1}}$ (dry weight) and the water concentration (C_{water}) in $\mu\mathrm{g\,L^{-1}}$. The mean log BAFs were calculated for every detected compound and are summarized in Tables 4 and 5. The obtained log BAF values varied between 0.6 $\mathrm{L\,kg^{-1}}$ for terbutylazine to 1.7 $\mathrm{L\,kg^{-1}}$ for metolachlor. Comparable experimental data are not available for the target analytes, indicating the relevance of this study. Besides the ecological relevance of BAFs, their determination is also important for regulatory purposes. Nowadays, the European regulation on chemical substances REACH requires bioconcentration factors (BCFs), which can be considered as BAFs

Table 6
Calculated water concentrations ($\mathrm{ng\,L^{-1}}$) of the detected pharmaceuticals and pesticides at eight stations in the Belgian coastal zone in 2008, 2009 and 2010 (n.d. = not detected).

	Sea	OO1		OO2		OO3			ZB1			ZB2			NP1			NP2		
	2009	2008	2010	2008	2009	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Pharmaceuticals																				
Propranolol	93	285	169	682	1169	443	348	1417	405	824	498	443	221	1855	6329	658	2095	7294	812	2663
Carbamazepine	21	136	149	322	587	170	302	650	147	280	225	161	83	367	200	222	455	269	166	732
Pesticides																				
Pirimicarb	n.d.	n.d.	n.d.	41	10	10	3	25	4	3	20	5	n.d.	n.d.	27	3	48	53	13	118
Simazine	n.d.	81	77	148	75	71	64	132	49	45	69	40	34	28	148	63	110	159	106	206
Isoproturon	12	28	n.d.	67	33	51	29	31	73	41	37	70	37	24	83	51	45	148	260	106
Diuron	6	143	32	263	92	103	80	70	120	56	36	262	68	73	75	73	47	197	93	104
Atrazine	n.d.	31	13	56	42	34	33	19	25	23	20	16	17	14	41	35	20	56	41	41
Terbutylazine	3	94	95	355	63	283	62	220	115	78	179	57	51	122	385	251	383	407	215	469
Metolachlor	1	19	4	104	7	49	5	21	22	8	19	30	5	8	113	8	38	164	22	82

obtained on a standardized manner [56], for all compounds. However, the experimental determination of BCFs is time-consuming, complicated, expensive and moreover, calculating these for the many thousands of chemical substances of interest is simply not possible [57]. Therefore, in recent years, QSARs have been developed to predict the partitioning of pollutants in biotic tissue [52–54]. These modeling QSARs may certainly form the subject for further research, to enable accurate estimations of the accumulation and toxicity caused by micropollutants in organisms, thereby reducing the experimental variability as much as possible.

3.5.2. Untargeted compounds

A major advantage of the use of Orbitrap MS, is its suitability for untargeted analysis [22]. In theory, an infinite number of analytes could be screened using the high-resolution full scan data. Thus, the presence in the sampler extracts of non-a priori selected pharmaceuticals and pesticides could be examined as well. The retrospective screening of the passive sampler extracts, using a 5 ppm window, revealed the presence of two pharmaceuticals, simvastatin and fluoxetine, and one pesticide, diazinon. Since no $K_{sa/wa}$ values were obtained for these compounds, estimation of TWA water concentrations was impossible. High affinity of simvastatin, fluoxetine and diazinon for the PDMS samplers could be expected, since their log K_{ow} values amounted to 5.19, 4.05 and 3.81, respectively [42]. Only a small selection of pharmaceuticals and pesticides were screened afterwards, so it can be assumed that still other pharmaceuticals and pesticides were present in the passive sampler extracts. In conclusion, the excellent applicability of a new analytical approach to quantify a limited number of rather polar micropollutants in PDMS samplers was presented. In this context, the present study is only the initial stage of a more comprehensive study. Indeed, future research must enable the quantification of a very wide group of pharmaceuticals and pesticides in PDMS samplers by the development of an extensive database including retention times, accurate masses and $K_{sa/wa}$ values.

4. Conclusions

PDMS passive sampling devices were evaluated as a monitoring tool for measuring the concentrations of a wide group of frequently used pharmaceuticals and intensively applied pesticides in marine waters. Therefore, a new extraction procedure using 1:1 methanol/acetonitrile was optimized and analysis was performed using ultra-high performance liquid chromatography coupled to high resolution Orbitrap MS. Detection with the Exactive™ Orbitrap MS enabled the use of a very narrow mass tolerance window of 5 ppm, providing high mass accuracy. These analytical procedures were validated successfully according to CD 2002/657/EC [27] and SANCO/10684/2009 [36], showing their excellent performance in quantifying pharmaceuticals and pesticides in PDMS passive sampler devices. In addition, an equilibrium-experiment was performed to determine the sampler-water partition coefficient ($K_{sa/wa}$) of the target analytes. Only a limited number of pharmaceuticals showed affinity for the PDMS samplers, while for most pesticides high $K_{sa/wa}$ values were obtained. Deployment of the passive samplers at five stations in the Belgian coastal zone revealed the presence of propranolol, carbamazepine and seven pesticides. Calculation of the water concentration resulted in very high levels of propranolol up to 7 $\mu g L^{-1}$, which is probably an overestimation due to the salting out effect. The concentrations of the other compounds were below 750 $ng L^{-1}$. These long-term and large-scale TWA concentrations provide appropriate information for assessing the pollution level of these pollutants in the marine environment, in particular with respect to their biological uptake.

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