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Investigation of the matrix effects on a HPLC-ESI-MS/MS method and application for monitoring triazine, phenylurea and chloroacetanilide concentrations in fresh and estuarine waters

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Abstract:

In this work, the effects of matrix interferences on the analytical performance of a new multiresidue method based on off-line solid phase extraction followed by reversed-phase liquid chromatographic separation and electrospray triple quadrupole mass spectrometric detection were investigated. This technique allows the simultaneous determination of 30 triazines, phenylureas and chloroacetanilides, extracted from freshwaters, in 40 minutes. Quantifications were performed with the use of appropriate internal standards (*i.e.* atrazine D5, diuron D6 and metolachlor D6). The limits of quantification were from 1 to 32 ng L^{-1} for the triazines, from 5 to 59 ng L^{-1} for the phenylureas and from 13 to 54 ng L^{-1} for the chloroacetanilides. The matrix effects were studied by spiking various waters (*i.e.* tap, river, pond and sea waters) with the chemicals of interest. The results showed that the samples with the highest conductivity (*i.e.* seawater) and the most abundant dissolved organic matter content (*i.e.* pond water) exhibited important matrix effects with signal suppressions and high imprecision, respectively. These matrix effects were strongly minimized by performing appropriate internal standardizations. Afterward, this analytical method was applied for analyzing environmental samples from either river or estuarine waters and for monitoring herbicide input in a freshwater-seawater interface.

Keywords: HPLC-ESI-MS/MS, multiresidue method, matrix effects, herbicides, natural water quality, river-estuarine interface

Introduction

Herbicides are representative of 40-45 % of the agricultural pesticide use in the world[1]. Regarding the herbicide legislation, there are some differences between US and European policies. For instance, the phenylureas are not used at all in US whereas several triazines and phenylureas are controlled or even forbidden in Europe. This is the case of the atrazine since it was completely banned from the agricultural use in many countries (e.g. Germany, Italy, Austria, Sweden, Norway and France). However, several triazines and phenylureas are authorized for the non-agricultural purposes which represent 22 % of the total herbicide use [1]. The European framework directive in the field of water policy 2000/60/EC [2] seek to prevent deterioration, to enhance and to restore bodies of surface water, to achieve good chemical and ecological status of such water and to reduce pollution from discharges and emissions of hazardous substances. Among these hazardous substances, the monitoring of herbicides such as atrazine, simazine, alachlor, diuron and isoproturon in freshwaters is imperative and there is a need for pertinent and accurate data to compare with current legislation and environmental quality standards [3]. Regarding to drinking water, the levels of the pesticide residues in natural waters is of public concern and the maximum concentration admissible for pesticides is 0.1 µg L^{-1} for individual compounds and 0.5 µg L^{-1} for the sum of them [4].

Several methods were developed for the simultaneous analysis of different herbicide classes. Multiresidue methods using GC/MS can be applied for the analysis of triazines [5, 6] and chloroacetanilides [6, 7] but the determination of thermally labile phenylureas is more delicate since the degradation products depend on the injection solvent composition [8]. Classical approaches are based on on-line or off-line solid-liquid extractions followed by HPLC-DAD analyses [9-12] but UV detection lack of specificity and both identifications and

quantifications can be difficult with complex matrices. More recent multiresidue methods involved HPLC separations coupled with electrospray mass spectrometric (ESI-MS) detections [10, 11, 13-20]. This technique allows the simultaneous determination of several herbicides with short analysis times but the purity of the samples must be considered. In fact, the response with electrospray ionization is affected by the polar/ionisable impurities which may be present in the matrix and perturb the ionization processes [21, 22].

This paper addresses two objectives with the investigation of the matrix effects for various types of water samples and the development of an accurate method based on ESI-MS/MS detection that will be used for monitoring several herbicides in these types of waters. This method consists in an improvement of a previous HPLC-DAD multiresidue technique based on an off-line solid phase extraction of several polar herbicides from freshwaters [9, 23]. The use of the ESI-MS/MS detection allowed the quantification of a larger number of compounds with a shorter analysis time. Nevertheless, a correction of the signal suppression (or enhancement) due to the matrix effects was necessary. Thus, the matrix effects were studied by spiking different natural waters with various conductivities and low or high dissolved organic matter contents as impurities. Afterwards, this multiresidue method was applied for monitoring herbicide concentrations in rivers and in a freshwater-seawater estuarine interface.

Experimental

Chemicals

Acetonitrile supragradient, methanol gradient and water gradient (HPLC grade) were purchased from ICS-SCIENCE Groupe (France). Oasis HLB cartridges (6 mL, 500 mg, 60 µm) were provided by Waters (France). GF/F filters (47 mm ∅) were provided by Whatman (France). All analytical standards were purchased from Dr. Ehrenstorfer (Germany): ametryn, atrazine, cyanazine, atrazine-desethyl (DEA), terbuthylazine-desethyl (DET), atrazine-desisopropyl (DIA), irgarol 1051, prometryn, propazine, simazine, terbuthylazine, terbutryn, chlortoluron, diuron, 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), 1-(3,4-dichlorophenyl)-urea (DCPU), fenuron, isoproturon, 1-(4-isopropylphenyl)-3-methylurea (IPPMU), 1-(4-isopropylphenyl)-urea (IPPU), linuron, metobromuron, metoxuron, monolinuron, monuron, neburon, acetochlor, alachlor, metolachlor, metazachlor, atrazine D5, DEA D6, diuron D6, metolachlor D6 and prometryn D6.

Solid phase extractions

Preconcentration of the analytes from water samples was accomplished by using solid-phase extraction (SPE) with Oasis HLB cartridges. This SPE procedure is adapted from previous works [9, 23]. Prior to SPE, 200 mL of water samples (pH adjusted to 7) were filtered using GF/F glass microfibre filters (0.7 µm pore size) and 10 µL of a stock solution (acetonitrile) containing 10 ng μL^{-1} of atrazine D5, diuron D6 and metolachlor D6 was added, resulting in fortification of the water samples with 0.5 µg L^{-1} of each internal standard. SPE was conducted using a VisiPrep 12-port manifold (Supelco, France). The conditioning,

extraction and rinsing steps were carried out under a 400 mm Hg vacuum (1 mmHg=133.322 Pa). The SPE cartridges were successively washed with 10 mL of methanol, conditioned with 10 mL of HPLC grade water, loaded with 200-mL water samples, then rinsed with 20 mL of HPLC grade water and dried with a stream of nitrogen for 30 minutes. Elutions were achieved with 5 mL of methanol. The 5-mL extracts were blown under a gentle stream of nitrogen and dissolved in 1 mL of an acetonitrile:water (10:90, v/v) mixture prior to the HPLC-ESI-MS/MS analyses. The final concentrations of the surrogates were about 100 µg L^{-1} after the solid phase extraction.

Evaluation of matrix effects

River and pond waters were collected in southwest part of France (Anan and Cestas, respectively). Seawater samples were collected in the Vilaine estuary, Brittany, France (Figure 1). Postextraction standard additions were performed for the evaluation of the matrix effects (Table 1) [24]. For this purpose, 3×200 mL of non fortified matrices (either tap, river, pond or sea water) were conditioned, filtered and preconcentrated using SPE as described previously. All the extracts were dried with nitrogen, spiked with 100 µg L^{-1} of both test chemicals and internal standards and then dissolved in 1 mL of an acetonitrile:water (10:90, v/v) mixture. In addition, one blank extraction of each matrix was done. Each blank was fortified with internal standards only and analyzed separately to determine the background concentrations.

Dissolved organic carbon measurements

Dissolved organic carbon (DOC) contents of each matrix were determined (Table 2). The water samples were filtered using GF/F glass microfibre filters (0.7 µm pore size) and the concentrations of DOC were measured using a model 1010 OI Analytical carbon analyzer with a 1051 auto-sampler (Bioritech, France). The total organic carbon analyses were performed with an high-temperature persulfate oxidation technology and according to the European standard ISO 8245:1999 [25].

HPLC separation

HPLC system: Finnigan SpectraSYSTEM SCM1000 Solvent Degasser, Finnigan SpectraSYSTEM P4000 Quaternary Pump, Finnigan SpectraSYSTEM AS3000 Autosampler (column oven set at 40°C) and Finnigan UV6000LP photodiode array detector (Thermo Electron Corporation**,** MA, USA). Detection wavelengths: λ=220 nm for ametryn, atrazine, cyanazine, DEA, DET, DIA, irgarol 1051, prometryn, propazine, simazine, terbuthylazine, terbutryn, acetochlor, alachlor, metolachlor and metazachlor, λ=240 nm for chlortoluron, diuron, DCPMU, DCPU, fenuron, isoproturon, IPPMU, IPPU, linuron, metobromuron, metoxuron, monolinuron, monuron, and neburon. The HPLC separation of triazines, phenylureas and chloroacetanilides (Figure 2) was performed with a Prontosil Spheribond ODS 2 column (150 x 4 mm, 3 µm) with a C_{18} (10 x 4 mm, 6 µm) guard column (Bischoff Chromatography, Germany). The injection volume and solvent composition were 50 µL and acetonitrile:water (10:90, v/v), respectively. The corresponding binary gradient composition is given in Table 3.

ESI-MS/MS detection

The HPLC system was coupled with an API 2000 (Applied Biosystems/MDS SCIEX, France) triple quadrupole mass spectrometer equipped with a turboionspray source (ESI). Optimization of source, gas and compound dependent parameters were achieved by infusing pure standard solutions (1 mg L^{-1} in acetonitrile:water mixtures) into the turboionspray source at a flow rate of 10 µL min^{-1} by using a syringe pump. The ionization mode was positive, the ion spray voltage was held at +5500 V and the declustering potential was optimized for each compound with voltages of about 20-30 V. The electron multiplier was set up to 2400 V. The nebulizing gas CG1 (N_2), the drying-gas CG2 (N_2) and the curtain gas (N_2) pressures were 45 psi, 80 psi and 40 psi, respectively. The CG2 temperature was set up to 500°C. CID product-ion spectra were acquired by colliding the Q1 selected precursor ions with N_2 (CAD=3 psi) and applying collision energies from 25 to 40 V in Q2. Both Q1 and Q3 were operated at unit resolution and the step size was m/z=0.1. The optimal multiple reaction monitoring (MRM) quantitative transitions of both test chemicals and internal standards are reported in Table 4. The parent ions of the MRM transitions correspond to the $[M+H]^+$ molecular peaks. Dwell times of 50 ms were used for each triazine or phenylurea whereas dwell times of 100 ms were used for each chloroacetanilide. A total dwell time of 1,9 s was used, resulting in a minimum of 12 data points for every chromatographic peak. Both external and internal calibration procedures were performed and the concentration ranges for the calibration curves were from 5 to 500 µg L^{-1} for the triazines and from 10 to 500 µg L^{-1} for both phenylureas and chloroacetanilides. 100 µg L^{-1} solutions of atrazine D5, diuron D6 and metolachlor D6 were used for the respective internal quantifications of triazines and their metabolites, phenylureas and their metabolites, and chloroacetanilides.

Results and discussion

Matrix spike experiments

It is well known that electrospray ionization suffers from matrix effects when polar/ionic compounds other than the analytes of interest, such as those originating from the sample matrix, are present [21, 22]. This phenomenon is attributed to competitive ionization of all of the appropriate species present in the sample [26]. The matrix effects may induce a loss of sensibility (i.e. ion suppression) and may well affect both accuracy and precision. Different methods can be used for overcoming the matrix effects: the complete removal of co-eluting substances by sample clean-up techniques such as gel permeation chromatography or solid phase extraction [16]. Such an approach is time consuming and difficult if the matrix is complex. Alternatively, the calibration standards can be make up in a matrix extract rather than in a pure solvent [19, 27]. The problem with this method is that the composition of such an extract cannot be guaranteed to be identical to that in which the chemical of interest must be determined [22]. Another common approach is based on the standard additions. Such a method provides both good accuracy and precision but the main disadvantage is that further analyses must be performed. Therefore, this approach is not suitable for daily and extensive analyses. Lastly, the use of internal standards would improve both accuracy and precision [17, 18, 27], but appropriate internal standards are sometimes not available. Concerning this work, different water matrices were spiked with the chemicals of interest for estimating the matrix effects. Some internal standards representative of three different herbicide classes (i.e. triazines, phenylureas and chloroacetanilides) were selected. Both internal and external calibrations were done and compared.

Triplicates of unfortified tap, river, pond and sea waters were preconcentrated using SPE. The matrices were spiked with both test chemicals and internal standards after solid phase extractions in order to eliminate the variability of the SPE recoveries and to estimate the matrix effects only. The seawater matrix analysis showed background concentrations lower than the limits of detection (LODs) for every compound. The analysis of the blank river matrix revealed background concentrations lower than the limits of quantification (LOQs; Table 4) for atrazine, DEA and simazine, and lower than the LODs for the other chemicals of interest. The analysis of the blank pond matrix showed background concentrations lower than the LOQs for diuron, fenuron, isoproturon and monuron, and lower than the LODs for the other chemicals of interest. Consequently, for each matrix, the contribution of the background concentrations was negligible (lower than 1 and 5 µg L^{-1} for the triazines and the phenylureas, respectively) in comparison to the concentrations of the fortifications (100 µg L^{-1}). The Table 1 gives the concentrations calculated with either external calibrations or internal calibrations. The spiking of tap and river waters with the test chemicals showed no significant differences between the internal and external calibrations (Table 1). There were also no peculiar matrix effects since the mean values of the triplicates were contained between 88 and 119 % of deviation (85-115 % for the internal calibrations). For the two matrices, the relative deviations from the expected 100 µg L^{-1} may be attributed to the instrumental uncertainty.

The HPLC-DAD analysis of both river and pond matrices spiked with the test chemicals may indicate the higher abundance of UV-absorbing organic matter in the pond water (Figure 2 a). The results of the dissolved organic carbon analysis (Table 2) confirmed this assumption with values of about 92 and 1.7 mg L^{-1} for the pond and river waters, respectively. Thus, the SPE purification did not eliminate all the dissolved organic matter (DOM) and regarding to the pond matrix, the concentrations were slightly overestimated with the external calibration (Table 1), especially for phenylureas and chloroacetanilides with

relative deviations between 106 and 128 % (except fenuron with 98 %). Steen et al. [28] observed some matrix effects such as ion suppression with the presence of DOM like humic acids. The authors performed external calibration and suggested the use of a tandem aminopropyl/LiChrolut EN SPE set-up for removing the humic acids. In our case, the SPE procedure was not modified for the pond matrix and the use of surrogates such as diuron D6 and metolachlor D6 minimized the matrix effects for phenylureas and chloroacetanilides, respectively. Actually, relative deviations of 100-114 % for both classes with the internal calibration (except DCPMU and fenuron with 120 and 92 %, respectively) were observed. For the triazines, the use of DEA D6 instead of atrazine D5 as internal standard for both DIA and DEA did not provide significant improvements of the results. Prometryn D6 was also tested for the quantification of the methylthiotriazines (i.e. irgarol 1051, prometryn, ametryn and terbutryn). The correction of the matrix effects was slightly better for some compounds (e.g. 112 % instead of 121 % for terbutryn) but the occurrence of non-deuterated prometryn (about 1 % of the internal standard as impurity) could be problematic for the measurement of this herbicide at trace level. Furthermore, the interfering DOM present in the pond sample matrix induced higher imprecision with the external calibrations than with the internal calibrations. For instance, with the external calibration, relative standard deviations were higher than 25 % for 11 compounds and the respective RSDs of some herbicides like cyanazine and IPPU were up to 43 %. Regarding to the internal calibration method, the RSDs were mostly $\leq$ 10 % and the highest value was observed for IPPU (24 %). Therefore, methods based on standard addition into such a matrix without internal standard corrections might result in imprecise quantifications.

The seawater matrix is characterized by a low DOC content and the highest conductivity (Table 2). Like the river water, the HPLC-DAD analysis of the seawater (not showed) revealed the low abundance of UV-adsorbing interfering compounds. The HPLC-

ESI-MS/MS analysis (Table 1) showed strong ion suppression effects for some triazines like atrazine (62 %), DEA (61 %), DET (59 %), simazine (52 %) and terbuthylazine (66 %) when only external standardizations were performed. Signal suppression or enhancement was reported by Gil-García et al. [29] concerning the ESI analysis of some pyrethroids in seawater. The authors attributed these matrix effects to the presence of salts and others ionic compounds in the ionization source and suggested a cleanup step during the SPE procedure. In our study, the use of appropriate surrogates clearly reduced the matrix effects since values from 82 % (simazine) to 116 % of deviation (DIA and the metoxuron) were observed. Lastly, the RSDs were slightly better and acceptable with the use of internal standards ($\leq$ 22 %).

Finally, it seems that internal standardization is necessary for a matrix with high DOM content (i.e. pond water) for a better precision. This approach is also useful and sufficient for improving the accuracy with some peculiar matrices (i.e. sea water).

HPLC-ESI-MS/MS multi-residue analysis and SPE recoveries

The SPE optimized recoveries obtained with tap waters fortified with either 0.1 µg L^{-1} (n=5) or 0.5 µg L^{-1} (n=10) of test chemicals are reported in Table 4. The lowest values were observed for DEA and metazachlor with 73 %, fenuron and metobromuron with 75 %, and simazine with 78 %. For all the other herbicides, recoveries were $\geq$ 80 %. As shown before, the matrix effects are low for the tap water, especially with the use of surrogates. Consequently, these values really correspond to the SPE recoveries and they are in good agreement with the previous results obtained by Carabias-Martínez et al. [11] for extracting neutral phenylureas and acidic sulfonylureas. Different volumes of methanol were used for the elution and there was no real improvement of the results over 5 mL of solvent[23]. The extractions carried out with the Oasis HLB cartridges showed good reproducibility for most of

301 the test chemicals (RSD ≤ 18 %). Only DET exhibited a really higher RSD (22 %).
302 Reproducibility of the SPE-HPLC-MS/MS method was carried out with the measurement of
303 the same material (tap water spiked at two different levels, Table 4) under changed conditions
304 of time (1 week between each extraction and analysis) and with different observers.

305 The limits of quantification (Table 4) and the limits of detection were determined by
306 diluting standard solutions until ratios of S/N=10 and S/N=3, respectively. The
307 chromatographic separation of the 30 test chemicals in 40 minutes is shown in Figure 2.
308 Carry-over effects were not observed with the ESI-MS/MS detection. The linear dynamic
309 ranges were from the LOQ to 500 µg L^{-1}. A replicate analysis (n=4) of the same sample (100
310 µg L^{-1} standard solution in an acetonitrile:water mixture) was performed and the relative
311 standard deviations (RSDs) of the ESI-MS/MS detection were ≤ 12 % for triazines, ≤ 10 %
312 for phenylureas and ≤ 9 % for chloroacetanilides.

313

314 **Solid phase extraction and HPLC-ESI-MS/MS analysis of fortified river water samples**

315

316 The results related to the spiking of river water with the test chemicals are reported in
317 Table 4. The samples were fortified at two different level concentrations (0.05 and 0.5 µg L^{-1}),
318 preconcentrated by using the SPE procedure and quantified with internal calibrations. The
319 data did not require corrections for the background concentrations as mentioned above. The
320 results showed that the relative deviations from the expected values were higher but
321 acceptable for the lowest concentration. In fact, most of the deviations did not exceed ± 30 %
322 except for cyanazine, DEA and metazachlor (about + 40 % with 69-70 ng L^{-1}). The relative
323 deviations became negligible at higher concentrations with values from 0.393 µg L^{-1} (IPPU)
324 to 0.641 µg L^{-1} (prometryn). Globally, the data showed that this multiresidue HPLC-ESI-

MS/MS method based on internal calibrations provides reliable quantitative results for river waters.

Application to the waters from the Vilaine river and estuary

Several water samples from the Vilaine river and estuary were collected between April 16th to June 12th 2007. As shown in Figure 1, the sampling sites were located in the river upstream (Arzal dam) and in the estuary (les Granges and Maresclé). The samples from the Arzal dam correspond to the freshwaters coming from the Vilaine river and the samples from les Granges and Maresclé are characteristic of a marine environment. The estuarine waters were collected at either low tide or high tide. During april 16th, the sample from Arzal contained only some traces of DEA (0.03 µg L^{-1}), diuron (0.05 µg L^{-1}), and isoproturon (0.12 µg L^{-1}) (Figure 3). Isoproturon is generally used for the winter wheat weeding. The analyses of waters from the estuary revealed the detection of some herbicides with concentrations lower than the LOQs.

On May 29th, the freshwater from Arzal showed concentrations of diuron and acetochlor of about 0.1 µg L^{-1}. The occurrence of acetochlor can be attributed to the agricultural treatments during the spring. Diuron is forbidden for the agricultural purposes in Brittany. However this compound is used as biocide in the antifouling paints [30] and its occurrence is probably due to the high sailing activity at the Arzal dam. At the same date, the analysis of the sample from Les Granges at low water revealed the occurrence of atrazine, DEA, simazine, diuron, isoproturon and acetochlor whereas the Maresclé (sample colleted during the low water as well) was less impacted by these chemicals (detection of metolachlor only). The sampling site of Les Granges is closer to the Arzal dam and, consequently, it should be more contaminated by the herbicides carried out by the freshwaters from the river.

Finally, strong dilutions (concentrations < LOQs) were observed for both Les Granges and Maresclé about 6 hours later (samples collected during the high tide).

The last sample (June 12th) from Arzal exhibited an increase of irgarol 1051 and diuron concentrations (0.19 µg L^{-1} and 0.24 µg L^{-1}, respectively). Both of these chemicals are contained in antifouling paints [30-32]. Some chloroacetanilides (alachlor, acetochlor and metolachlor) were detected but their concentrations did not exceed 0.1 µg L^{-1}. Regarding to current environmental quality standards (EQS) [3] applicable to surface and coastal water [33], the relatively short period of the study (3 sampling campaigns in two months) makes the comparison with the annual average (AA) EQS difficult. Only the concentration of the diuron on June 12th exceeded the AA-EQS with 0.24 µg L^{-1} (Arzal dam) but this value was really lower than the maximum allowable concentration (MAC) EQS (1.8 µg L^{-1}). Consequently, if only the results obtained during the couple of months are considered, it might be assumed that the chemical pollution should not disturb the aquatic ecosystems of both Vilaine river and Arzal dam for this period. Our results showed also an input of several herbicides in the estuary but, at our knowledge, there are no legislative limits for some pollutants (i.e. irgarol 1051, acetochlor and metolachlor) in coastal water.

Unlike to the results obtained on May 29th, very low concentrations were detected in the marine environment at both low and high waters (only some traces of diuron and simazine in Les Granges) on June 12th (Figure 3). This decrease might be attributed to a lower contribution of freshwaters since the weekly average flow rates of the Vilaine river at the Arzal dam were about 70.6 $m^3.s^{-1}$ and 44.7 $m^3.s^{-1}$ before May 29th and June 12th, respectively. However, the daily amount estimates of some herbicides like diuron and irgarol 1051 were higher on June 12th (770 g and 610 g, respectively) than on may 29th (520 g and non-detected, respectively). In the same way, the large daily amount of isoproturon (570 g) carried by the Vilaine river on April 16th was not revealed within the estuarine waters. Thus, it appears that

hydrometric data associated to a grab sampling may not explain the behaviour of the herbicides in this estuary. Such a hydrosystem is characterized by strong dilution and dispersion phenomena due to the tides. In this case, grab samples give only a snapshot of the water contamination level and more reliable concentration estimates could be achieved with the use of polar organic chemical integrative samplers [23, 34]. Actually, such devices allow the determination of time-weighted average (TWA) concentrations, which is a fundamental part of an ecological risk assessment process for chemical stressors and the further determination of EQS for marine waters.

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392 **TABLES**

393 Table 1. Determination of the matrix effects with 200 mL of various waters (n=3 for each
394 matrix) preconcentrated by using SPE and then spiked with 100 µg L^{-1} of the test chemicals
395 (triplicates). Values expressed in % of deviation from the expected concentrations (% RSD).

396

Herbicides	Tap water		River water		Pond water		Sea water	
	E.S. [a]	I.S. [a]	E.S.	I.S.	E.S.	I.S.	E.S.	I.S.
Ametryn	103 (5)	99 (2)	100 (9)	108 (6)	99 (6)	104 (15)	89 (5)	105 (15)
Atrazine	108 (4)	100 (8)	93 (6)	100 (5)	104 (24)	107 (1)	62 (8)	96 (2)
Cyanazine	88 (18)	91 (8)	103 (3)	111 (4)	126 (43)	124 (11)	104 (17)	115 (9)
DEA	98 (1)	91 (3)	99 (4)	106 (8)	90 (28)	91 (8)	61 (6)	99 (3)
DET	93 (6)	85 (8)	95 (8)	103 (6)	103 (30)	105 (5)	59 (8)	99 (1)
DIA	94 (6)	88 (4)	90 (14)	96 (18)	88 (34)	88 (13)	83 (10)	116 (4)
Irgarol 1051	100 (7)	92 (9)	94 (6)	102 (5)	118 (8)	119 (16)	94 (10)	112 (15)
Prometryn	101 (4)	98 (3)	101 (9)	109 (7)	108 (7)	112 (16)	93 (4)	104 (18)
Propazine	98 (1)	93 (7)	90 (9)	96 (9)	124 (32)	122 (9)	66 (10)	103 (2)
Simazine	103 (9)	96 (12)	96 (6)	105 (4)	98 (20)	101 (5)	52 (5)	82 (2)
Terbuthylazine	97 (5)	89 (9)	90 (11)	97 (11)	112 (25)	119 (8)	66 (11)	102 (5)
Terbutryn	98 (8)	94 (5)	95 (6)	103 (4)	116 (13)	121 (13)	92 (6)	101 (20)
Chlortoluron	102 (6)	97 (6)	96 (1)	101 (5)	119 (25)	111 (10)	104 (12)	113 (9)
Diuron	110 (13)	104 (13)	101 (2)	106 (5)	126 (17)	112 (5)	86 (12)	103 (5)
DCPMU	119 (3)	113 (4)	92 (7)	97 (6)	128 (20)	120 (8)	89 (9)	106 (9)
DCPU	92 (13)	94 (11)	93 (2)	98 (3)	124 (39)	114 (20)	78 (13)	96 (3)
Fenuron	103 (6)	98 (6)	91 (8)	95 (12)	98 (22)	92 (9)	103 (4)	107 (22)
Isoproturon	111 (6)	104 (6)	93 (4)	97 (6)	114 (22)	108 (8)	123 (7)	113 (17)
IPPMU	105 (9)	100 (9)	95 (1)	99 (5)	117 (30)	110 (13)	102 (13)	114 (10)
IPPU	105 (18)	100 (11)	100 (5)	104 (1)	122 (43)	113 (24)	97 (18)	111 (7)
Linuron	115 (11)	109 (11)	106 (2)	111 (6)	108 (11)	103 (7)	82 (25)	101 (15)
Metobromuron	106 (5)	100 (5)	99 (6)	102 (4)	111 (16)	105 (6)	89 (19)	108 (13)
Metoxuron	118 (11)	112 (11)	96 (1)	101 (2)	118 (26)	107 (14)	111 (10)	116 (14)
Monolinuron	92 (7)	89 (4)	107 (7)	111 (5)	106 (17)	100 (1)	86 (10)	105 (11)
Monuron	96 (12)	92 (12)	91 (4)	107 (1)	111 (17)	104 (11)	106 (12)	113 (9)
Neburon	109 (16)	102 (14)	94 (2)	99 (6)	115 (22)	109 (6)	91 (9)	109 (14)
Acetochlor	114 (12)	104 (8)	89 (5)	101 (7)	122 (15)	106 (2)	92 (21)	89 (6)
Alachlor	96 (2)	90 (13)	95 (3)	106 (7)	117 (12)	101 (6)	79 (12)	82 (12)
Metolachlor	105 (12)	95 (6)	92 (1)	104 (6)	115 (21)	100 (4)	108 (30)	106 (9)
Metazachlor	116 (5)	109 (10)	106 (4)	115 (9)	122 (24)	104 (5)	109 (26)	110 (11)

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398 [a] Quantifications with either external (E.S.) or internal (I.S.) standardizations.
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401 Table 2. Physicochemical properties of the different matrices.

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Parameters	Tap water	River water	Pond water	Sea water
Conductivity (μS cm^{-1})	423	199	228	53300
pH	7.35	7.67	6.63	7.81
DOC (mg L^{-1}) [a]	0.45±0.01	1.69±0.03	92.35±4.23	1.30±0.04

403
404 [a] Values ± 1 S.D.

405

406

407 Table 3. Linear gradient composition (A: acetonitrile, B: water) for the separation of triazines,
408 phenylureas and chloroacetanilides on a reversed-phase column (Spheribond ODS 2, 150 x 4
409 mm, 3 μm).

410
411

Time (min)	% A	% B	Flow rate (mL min^{-1})
0	10	90	0.5
2	10	90	0.5
18	45	55	0.5
30	80	20	0.6
33	80	20	0.6
36	10	90	0.5
40	10	90	0.5

412

413

414 Table 4. LC-ESI-MS/MS and SPE optimized parameters for the herbicide analysis (2 level
415 fortification of tap water). Validation of the method with the fortification of a river water with
416 either 0.05 or 0.5 µg L^{-1}.

Peak numbers	Herbicides	MRM transitions	Surrogates	Recoveries [a] (% RSD)	LOQ [b] (ng L^{-1})	River water [c] spiked with 50 ng L^{-1}	River water [c] spiked with 500 ng L^{-1}
1	Ametryn	228>186	Atrazine D5 (221>179)	96 (13)	1	58	586
2	Atrazine	216>174	Atrazine D5	96 (12)	3	52	525
3	Cyanazine	241>214	Atrazine D5	104 (11)	23	69	436
4	DEA	188>146	Atrazine D5	73 (14)	7	69	585
5	DET	202>146	Atrazine D5	105 (22)	3	52	427
6	DIA	174>104	Atrazine D5	80 (13)	32	59	515
7	Irgarol 1051	254>198	Atrazine D5	112 (9)	1	60	544
8	Prometryn	242>158	Atrazine D5	98 (11)	1	62	641
9	Propazine	230>146	Atrazine D5	105 (15)	3	48	446
10	Simazine	202>132	Atrazine D5	78 (13)	9	53	625
11	Terbuthylazine	230>174	Atrazine D5	103 (13)	3	49	488
12	Terbutryn	242>186	Atrazine D5	110 (8)	1	50	545
13	Chlortoluron	213>72	Diuron D6 (239>78)	80 (11)	9	53	632
14	Diuron	233>72	Diuron D6	80 (14)	15	53	602
15	DCPMU	219>127	Diuron D6	102 (8)	49	43	450
16	DCPU	205>127	Diuron D6	122 (16)	46	39	397
17	Fenuron	165>72	Diuron D6	75 (10)	8	49	520
18	Isoproturon	207>72	Diuron D6	82 (8)	5	66	477
19	IPPMU	193>94	Diuron D6	85 (12)	19	52	544
20	IPPU	179>137	Diuron D6	99 (12)	43	49	393
21	Linuron	249>160	Diuron D6	88 (10)	54	42	520
22	Metobromuron	259>170	Diuron D6	75 (11)	59	61	639
23	Metoxuron	229>72	Diuron D6	87 (11)	8	54	552
24	Monolinuron	215>126	Diuron D6	92 (6)	38	60	487
25	Monuron	199>72	Diuron D6	85 (11)	25	57	555
26	Neburon	275>88	Diuron D6	107 (18)	44	45	483
27	Acetochlor	270>224	Metolachlor D6 (290>258)	91 (17)	53	52	454
28	Alachlor	270>238	Metolachlor D6	97 (14)	54	44	526
29	Metolachlor	284>252	Metolachlor D6	95 (18)	15	50	525
30	Metazachlor	278>134	Metolachlor D6	73 (13)	13	70	606

417
418 [a] SPE recoveries for tap water spiked with either 0.1 µg L^{-1} (n=5) or 0.5 µg L^{-1} (n=10). Quantifications were
419 carried out with internal standardizations.
420 [b] LOQ at S/N=10 after SPE preconcentrations.

[c] Concentrations calculated with the solid phase extractions of 200 mL of river water spiked with the test chemicals. Quantifications were carried out with internal standardizations.

FIGURES

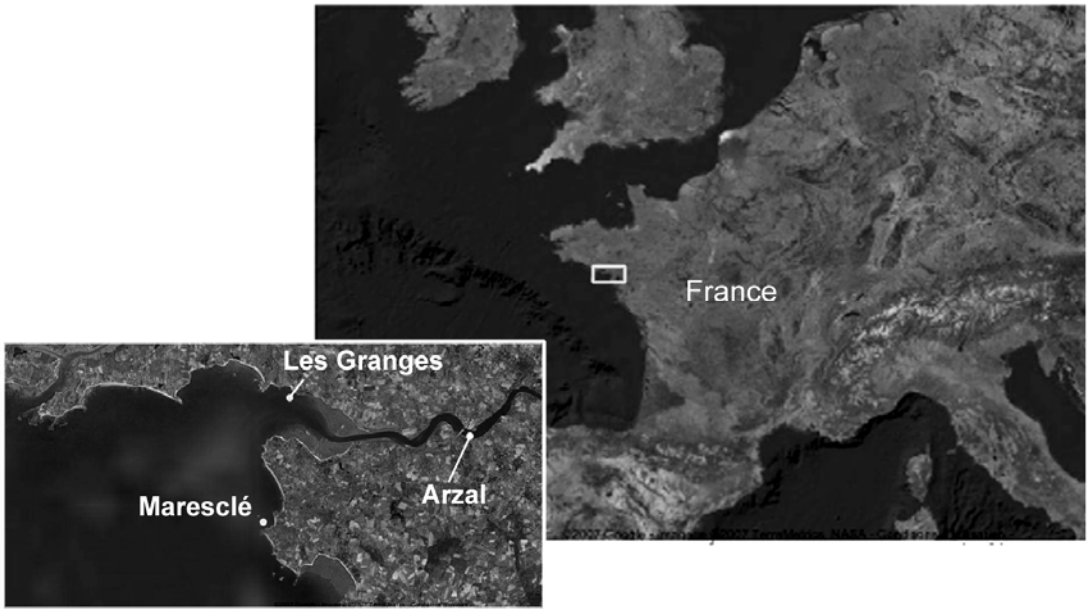
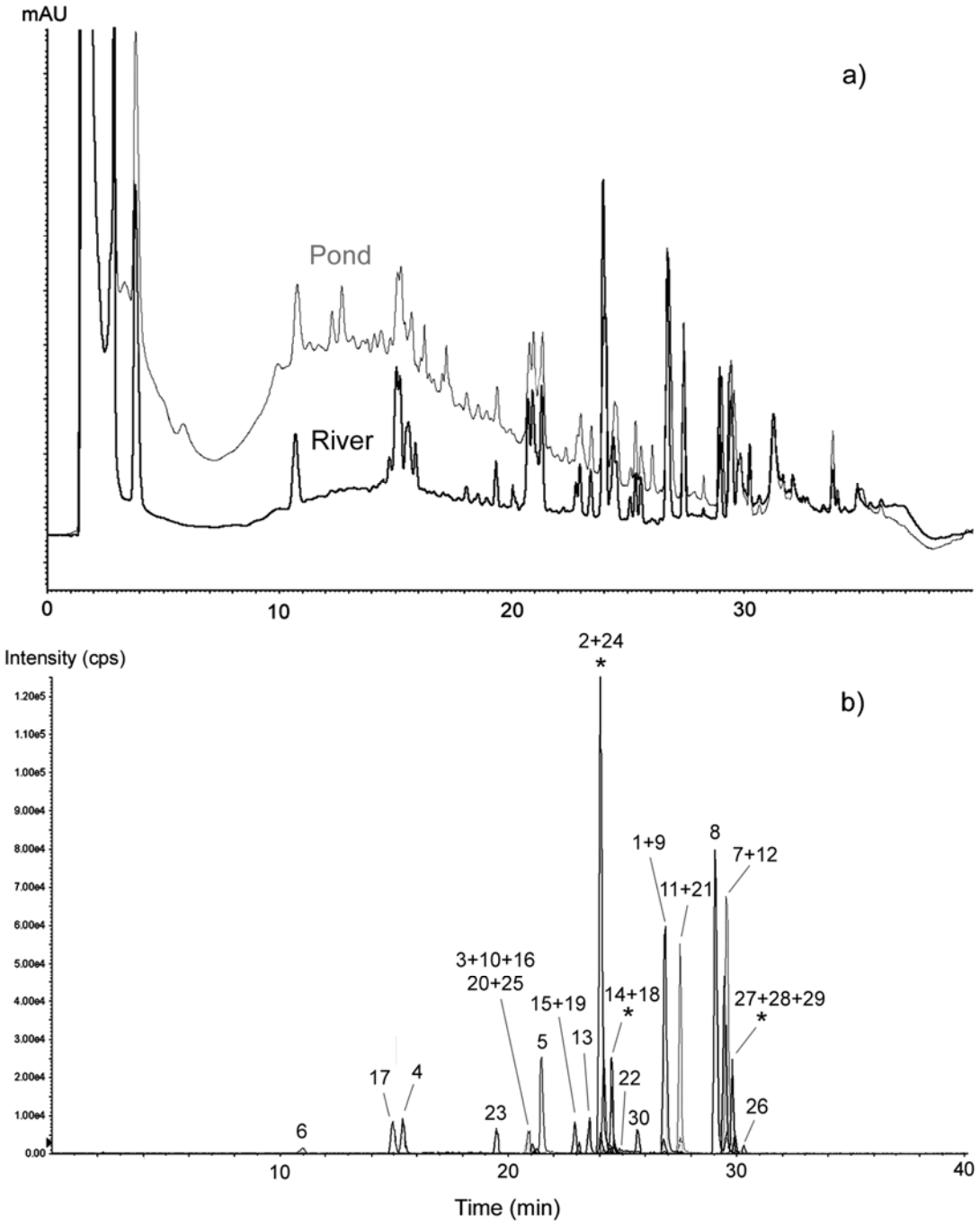


Figure 1. Location of the Vilaine estuary (Brittany, France) and the 3 sampling sites (Arzal dam, Les Granges and Maresclé).



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436

437 Figure 2. (a) HPLC-DAD (λ=220 nm) analysis of a 50 µg L^{-1} standard mixture in either pond or
438 river matrix. (b) HPLC-ESI-MS/MS analysis of a 10 µg L^{-1} standard mixture in HPLC grade
439 acetonitrile:water (20:80, v/v) mixture. Peak number attributions are reported in Table 4. (*)
440 Internal standards: atrazine D5, diuron D6 and metolachlor D6.

441

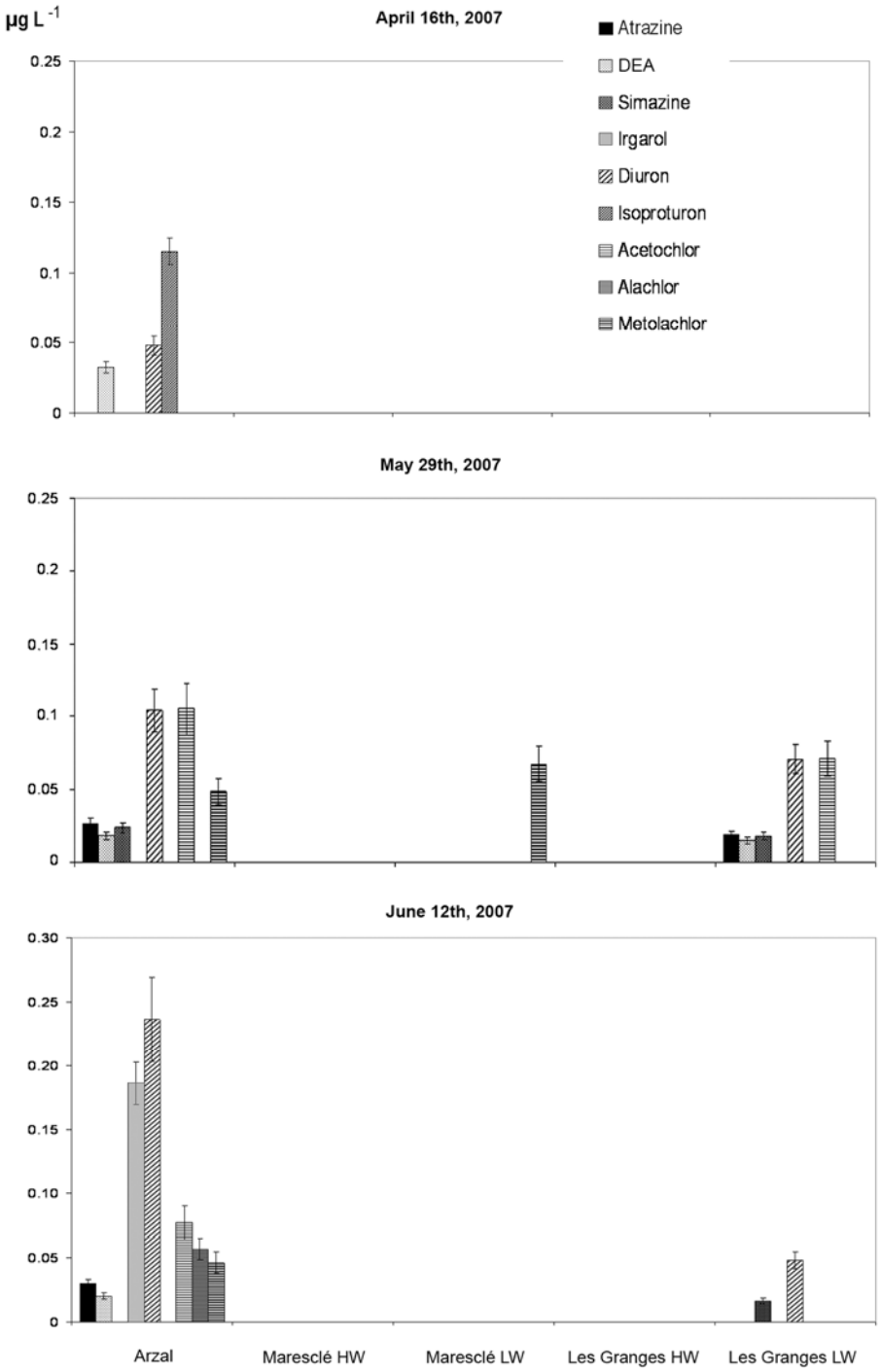

Figure 3. Concentrations (µg L^{-1}) of the different herbicides in the water samples from the Arzal dam and the Vilaine estuary (Les Granges and Maresclé). The samples were collected at either low (LW) or high water (HW).

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