Journal of Chromatography A, 1218 (2011) 1492–1502

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Liquid chromatography coupled with tandem mass spectrometry method for thirty-three pesticides in natural water and comparison of performance between classical solid phase extraction and passive sampling approaches

Sophie Lissalde[a,c,*], Nicolas Mazzella[a,**], Vincent Fauvelle[a], François Delmas[a], Patrick Mazellier[b,c], Bernard Legube[c]

[a] CEMAGREF, REBX Unit, 50 Avenue de Verdun, 33612 Cestas, France
[b] Université de Bordeaux, CNRS, UB1, UB4, UMR 5255, ISM-Laboratoire de Physico et Toxico Chimie de l'Environnement, Talence F-33405, France
[c] Université de Poitiers, CNRS – UMR 6008, Laboratoire de Chimie et Microbiologie de l'Eau, ESIP, Poitiers, France

ARTICLE INFO

Article history:
Received 20 July 2010
Received in revised form 3 January 2011
Accepted 15 January 2011
Available online 22 January 2011

Keywords:
SPE
POCIS
LC–ESI-MS/MS
Pesticides
Matrix effects
Water

ABSTRACT

The aim of this study is to propose an analytical method for determining different classes of pesticides in water using LC–ESI-MS/MS. Two techniques of field-sampling and analyte extraction were used: solid phase extraction (SPE) of water samples from active sampling and field exposure of Polar Organic Chemical Integrative Samplers (POCIS). We have worked with thirty-three molecules representing eight pesticide classes: carbamates, chloroacetanilides, dicarboximides, morpholines, organophosphorous, phenylureas, strobilurines and triazines. First, liquid chromatography separation protocols and the optimization of the ESI-MS/MS parameters were developed. Then, the SPE step was optimized to obtain acceptable levels of recovery for the various classes of molecules. The matrix effect that may significantly lower the ionization efficiency with ESI interfaces was evaluated and minimized. The performances (limits of quantification, accuracy and precision) of the SPE and POCIS techniques were evaluated, and a comparison between the active and passive sampling techniques was carried out with a field application.

1. Introduction

With the implementation of the European Water Framework Directive [1], we need reliable, efficient and low-cost methods for monitoring freshwater quality. Pollution from pesticides is not only problematic for the human health but also for aquatic organisms. Nowadays, pesticide residues are found in all surface waters and in a growing number of aquifers. Different national and international regulations impose increasingly strict controls of natural and drinking waters. To this end, we have developed a high performance liquid chromatography–electrospray-tandem mass spectrometry (HPLC–ESI-MS/MS) multiresidue method to monitor different classes of molecules in a single run with acceptable sensitivity and the assurance of obtaining no false positives [2].

The method was developed for the analysis of water samples after a solid phase extraction (SPE) step but also for the analysis of Polar Organic Chemical Integrative Sampler (POCIS) [3] extracts. Therefore, the instrumental method allows comparison of the two sampling/sample preparation techniques. Development of an analytical method for POCIS extract is important since passive sampling techniques might give more representative results of *in situ* pollution compared to active sampling such as grab sampling [4]. The POCIS device allows the concentration of large volumes of water, resulting in trace level detection, and smoothed integrative sampling over periods ranging from a week up to a month [4]. POCIS are continuously immersed in water, and thus integrate the pollution events occurring throughout the exposure period, providing time-weighted average concentration (TWAC) estimates with limits of quantification significantly lower than those obtained with the classical extraction of water samples [5]. For this purpose, we need to determine sampling rates and a calibration of POCIS was performed. LC–ESI-MS/MS is a well known technique for monitoring traces of pesticides in water [6]. Nevertheless, the performance of LC–ESI-MS/MS is generally tainted by matrix effects [7]. The recent developments and the improvement of liquid chromatography performance have led to faster analysis (e.g. the use of narrow bore and short columns, sub 2 μm particles, etc.). Matrix effects are well-known and widely documented in LC–ESI-MS/MS analyses. Studies essentially concern complex

* Corresponding author at: CEMAGREF, Unite de Recherche REBX, 50 Avenue de Verdun, 33612 Cestas, France. Tel.: +33 557 892 718.
** Corresponding author. Tel.: +33 557 892 718.
E-mail addresses: sophie.lissalde@cemagref.fr (S. Lissalde), nicolas.mazzella@cemagref.fr (N. Mazzella).

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doi:10.1016/j.chroma.2011.01.040

solid matrices like plant material, fruits, vegetables, fish or meat but such effects may also occur with water samples [8]. Matrix effects may affect a large range of molecules, especially the most polar when separation on a reversed-phase column is performed – depending on both the molecule's characteristics and the matrix composition. Different compounds may cause these matrix effects: salts, ion-pairing agents, endogenous compounds, metabolites, and proteins [9]. With HPLC, matrix interfering components are frequently co-eluted with the analyte peaks resulting in inaccurate quantification of these molecules due to ion suppression or signal enhancement [10]. The most polar compounds which exhibit the shortest retention times on a reversed-phase column are usually the most strongly impacted.

A large part of this work deals with the examination and subsequent minimization of these so-called matrix effects. Matrix effects were studied with the SPE of different natural water samples and also with POCIS exposed for 14 days in a river. Furthermore, the passive sampling approach was compared with classical water analysis (grab or repetitive and automated sampling) in terms of quantification limits, sample treatment and processing, and sampling representativeness of pesticide pollution.

2. POCIS theory

The use of passive sampling devices might give more representative results concerning the monitoring of pollutants in different environments like water, air or soil quality monitoring. One of the main interests of passive sampling is the high pre-concentration capacity of devices which allows detecting chemicals at ultra-trace levels. The devices are also continuously immersed in the water. Passive sampling techniques have been developed for the past 15 years [4,11–13] leading to various devices being put on the market like semipermeable membrane devices (SPMDs) [14,15], chemcatchers [16–18], diffusive gradient in thin film (DGT) [19] and POCIS [3]. All have different application domains [4,13,20,21]. This study deals with the use of the POCIS device for sampling polar organic molecules ($\log K_{ow} < 5$) in freshwater. Accumulation of pesticides in passive samplers like the POCIS is integrative with the assumption of isotropic exchange and when the sampling is performed during the linear uptake phase (during approximately one month depending on molecules). In this case, the TWAC of each analyte in water can be estimated with:

$$\overline{C}_w = \frac{m}{R_s t} \quad (1)$$

where m (μg) is the amount of the analyte accumulated in the receiving phase of a passive sampler after an exposure time t (days) and the sampling rate R_s ($L\,d^{-1}$). The values of R_s were molecule-dependent and we have previously determined the R_s for 33 pesticides in microcosms during laboratory experiments [22].

Passive samplers are commonly affected by the environmental conditions (e.g. biofouling, flow rate, temperature) and one of the most recent methods to overcome this limitation is the use of PRCs. A PRC is a compound that has moderate to high fugacity from the passive sampler sorbent, which does not interfere with the sampling and analytical processes and which is added to the device receiving phase prior to deployment [14,17]. A previous study has dealt with a field application of a PRC for POCIS where the relevance was discussed [23]. Under conditions of isotropic exchange, the elimination rate constant k_e of a PRC from the passive sampler sorbent can be determined with the following first-order relationship:

$$k_{e\,PRC} = \frac{\ln(C_{PRC\,0}/C_{PRC(t)})}{t} \quad (2)$$

where $C_{PRC(t)}$ is the residual concentration ($\mu g\,g^{-1}$) of PRC in the receiving phase after an exposure time (t) and $C_{PRC\,0}$ is the concentration of PRC spiked into the receiving phase before the exposure. The elimination rate constant of the same PRC is determined under both calibration ($k_{e\,PRC\,cal}$) and field ($k_{e\,PRC\,in\,situ}$) conditions, and an environmental adjustment factor (*EAF*) can be determined as follows:

$$EAF = \frac{k_{e\,PRC\,in\,situ}}{k_{e\,PRC\,cal}} \quad (3)$$

This *EAF* is applied to the calibrated sampling rates ($R_{s\,cal}$) and provides an estimate of the field sampling rates ($R_{s\,in\,situ}$) (Eq. (4)).

$$R_{s\,in\,situ} \approx R_{s\,cal} \times EAF \quad (4)$$

3. Experimental

3.1. Reagents and standards

The solvents (HPLC grade) were obtained from Sharlau (Sentmenat, Spain) except ethyl acetate, which was purchased from Fluka (St. Louis, MO, USA). Ultrapure water (UPW) was produced by a Synergy UV system from Millipore (Billerica, MA, USA). All eluents were filtered through 0.45 μm regenerated cellulose filters from Whatman. Ammonium acetate was purchased from Fluka. The pesticides selected for the study were: acetochlor, alachlor, atrazine, azoxystrobin, carbaryl, carbendazim, carbofuran, 3-hydroxycarbofuran, chlorfenvinphos, chlorpyriphos, chlortoluron, 1-(3,4-dichlorophenyl)-3-methyl urea (DCPMU, metabolite of diuron), 1-(3,4-dichlorophenyl)urea (DCPU, metabolite of diuron), desethylatrazine (DEA, metabolite of atrazine), desethylterbuthylazine (DET, metabolite of terbuthylazine), desisopropylatrazine (DIA, metabolite of atrazine), dimethoate, dimetomorph, hexazinon, 1-(4-isopropylphenyl)-3-methyl urea (IPPMU, metabolite of isoproturon), 1-(4-isopropyl phenyl) urea (IPPU, metabolite of isoproturon), isoproturon, linuron, metazachlor, methomyl, metolachlor, metoxuron, pyrimicarb, simazine, terbuthylazine, thiodicarb. Their analytical standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with a purity higher than 95.5%. Eleven internal standards or surrogates were used: atrazine-*d*5, carbaryl-*d*3, carbofuran-*d*3, chlorpyriphos-*d*10, DEA-*d*6, diuron-*d*6, methomyl-*d*3, metolachlor-*d*6, monuron-*d*6, prometryn-*d*6 and simazine-*d*5; all were also obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with a purity higher than 95.5%. And the Performance Reference Compound used in this study was DIA-*d*5.

Pesticide stock solutions were prepared in acetonitrile, stored at −18 °C for six months. The concentration was 100 mg L^{-1}. A working solution (1 mg L^{-1}) was prepared with a dilution of the stock solution in acetonitrile. This solution was also renewed every six months. The six calibration solutions were prepared in ultrapure water/acetonitrile, 90:10 (v/v) with concentrations ranging from 1 to 50 μg L^{-1}. Two solutions of deuterated labeled molecules were also prepared. A surrogate solution was made up of prometryn-*d*6 (1 mg L^{-1}), monuron-*d*6 (10 mg L^{-1}) and simazine-*d*5 (20 mg L^{-1}). The internal standard solution was composed of atrazine-*d*5, carbaryl-*d*3, carbofuran-*d*3, DEA-*d*6, diuron-*d*6, methomyl-*d*3 and metolachlor-*d*6 at 10 mg L^{-1}, and chlorpyriphos-*d*10 at 30 mg L^{-1}.

3.2. Automated sampler

Full-size Portable Samplers 6712 (Teledyne ISCO, USA) were used for determining the TWACs. The automatic water samplers were operated with a uniform time sampling mode. The sampling frequency and volume were hourly and 50 ± 5 mL, respectively. The TWACs were obtained with the accumulation of hourly samples

in a 19 L glass bottle. The glass bottles were kept in the dark and collected every week during the exposure period.

3.3. Solid phase extraction (SPE)

Four different SPE cartridges were evaluated: Oasis® HLB 500 mg, 5 mL, 50 μm, Oasis® HLB 150 mg, 5 mL, 50 μm, Oasis® HLB 60 mg, 3 mL, 50 μm from Waters and Chromabond HR-X 60 mg, 3 mL, 85 μm from Macherey-Nagel. The SPE step was performed with Visiprep™ and Visidry™ systems from Supelco. The SPE experiments were performed as follows: 100 mL of water sample were filtered on 0.7 μm GF/F filters (Whatman), the pH was adjusted to 7 and 10 μL of the surrogate solution were added prior to the extraction step. The cartridges were conditioned with 5 mL of methanol (MeOH) and 5 mL of UPW. Then, 50 mL of water sample were passed through the cartridge and 5 mL of UPW (with 5% of MeOH) were used for washing the cartridge. SPE cartridges were dried for 15 min under a gentle nitrogen stream and stored at 4 °C before the elution step. The elution was done during the month after elution if possible and was performed with two volumes of 3 mL, firstly 100% MeOH, and then MeOH:ethyl acetate 75:25 (v/v). These volumes and solvent choices were also optimized during the development of the method but results were not shown in this manuscript. The presence of ethyl acetate was necessary to improve the desorption of non polar molecules like chlorpyriphos. Finally, 10 μL of internal standard cocktail was added and the mixture evaporated to dryness. Following the analytical protocol, the sample extract was reconstituted in 1 mL of the initial HPLC eluent mixture (UPW:acetonitrile 90:10 (v/v)).

3.4. Polar Organic Chemical Integrative Sampler

The POCIS is composed of a sorbent (Oasis HLB powder) maintained between two polyethersulfone membranes. "Pharmaceutical" POCIS was used according to the results of a previous study [22]. Two compression holder washers are used to seal the device to prevent any loss of sorbent. Prior assembling the POCIS device, the sorbent was spiked with a PRC, DIA-*d5*. This provides quite acceptable *in situ* calibration. It leads to an improvement of the water concentration estimates obtained using this type of sampler by taking into account sampling variations due to environmental exposure conditions such as flow velocity, biofouling and temperature [23]. For the PRC spiking, 20 μg of DIA-*d*5 was dissolved in 25 mL of methanol. This solution was added to 5 g of Oasis HLB bulk sorbent and sonicated for 5 min. The solvent was removed with a rotary evaporator and the sorbent dried at 60 °C for 1 h. This procedure provided 5 g of Oasis HLB bulk sorbent spiked with about 4 $\mu g\,g^{-1}$. For each triplicate of POCIS exposed in the freshwater, three reference cartridges (3 mL empty polypropylene SPE tubes and polyethylene frits (PE)) were simultaneously prepared with 200 mg of sorbent containing the 4 $\mu g\,g^{-1}$ of PRC. These references were used for determining both the initial spike concentration and homogeneity. A blank POCIS was also used as a field and laboratory control. After exposure, each POCIS was opened and the solid sorbent phase (i.e. Oasis HLB powder) was recovered in a 50 mL glass beaker with 2 mL × 20 mL of ultrapure water. The sorbent was transferred into a 3 mL empty SPE tube with PE frit and packed under vacuum by using a Visiprep SPE Manifold. Afterwards, another PE frit was added to the top of the SPE cartridge. All the cartridges were dried under a gentle stream of nitrogen for 30 min. Analytes were eluted with two 3-mL volumes of eluent: firstly 100% MeOH, then MeOH:ethyl acetate, 75:25 (v/v) [22].

Table 1
Linear gradient of solvent composition.

Time (min)	Flow rate ($\mu L\,min^{-1}$)	ACN (%)	Ultrapure water + 5 mM ammonium acetate (%)
0	400	10	90
1	400	10	90
4	400	30	70
8	400	40	60
9.5	400	80	20
10.5	400	80	20
11	400	10	90
15	400	10	90

3.5. High performance liquid chromatography (HPLC) and electrospray and tandem mass spectrometry (ESI–MS/MS)

A HPLC Ultimate 3000 apparatus from Dionex was used (solvent rack SRD-3600 6 degasser channels, DGP-3600 M pump, WPS-3000 TSL Micro autosampler, TCC-3100 HP 1xRH 2P-6P thermostated column oven). Acetonitrile and 5 mM ammonium acetate solution were used with an analytical gradient of 15 min (Table 1).

Chromatographic separation was performed with a Gemini-NX C18 3 μm, 110 Å, 100 mm × 2 mm with a SecurityGuard cartridge Gemini-NX C18 4 mm × 2.0 mm, both from Phenomenex.

The detector was a mass spectrometer: an API 2000 triple quadrupole from Applied Biosystems/MDS/SCIEX. It was equipped with an electrospray ionization source (ESI) that was operated in the positive ionization mode. Mass acquisition was performed in the sSRM mode. Two transitions were registered for each molecule except for internal standards and surrogates. One of the SRM transitions was used in the quantification process and the other transition was used to confirm the molecule's presence and to avoid any false positives (Table 2). Parameters from the mass spectrometer (declustering potential – DP, collision energy – CE and cell exit potential – CXP) were optimized and the values are reported in Table 2. Analyst software 1.5.1 was used with the SRM algorithm for acquiring and interpreting the results. Scheduled MRM™ Algorithm works by reducing the number of concurrent MRMs during an LC gradient. Scheduled MRM™ Algorithm divides that task into smaller batches, by programming the instrument to look for each ion only when it is expected to enter the instrument from an upstream liquid chromatography system. It can greatly improve precision, accuracy, signal-to-noise, and throughput. Calibration was performed by a 6-point linearity range from 1 to 50 $\mu g\,L^{-1}$.

3.6. POCIS calibration

A calibration experiment was done in a tank of 80 L of tap water spiked with the pesticide cocktail at 1 $\mu g\,L^{-1}$. A previous study of pesticide loss in the tank was done (results not shown) and has shown the necessity of a second spike at the day 12 in order to keep the concentration approximately constant. POCIS were immersed and one POCIS was taken for analysis every 6 days over a 24 days period. The microcosm experimental design is described in an earlier work [22].

3.7. Matrix effects evaluation

The SPE cartridges Chromabond HR-X gave the best results in terms of pesticide recovery (see Section 4.1). Therefore matrix effects were evaluated using this type of cartridge on three different matrices: tap water, pond water from Cestas (south-west France) and river water (*Boutonne* river, south-west France). 50 mL of water were extracted with SPE cartridges (Chromabond HR-X) and the extracts spiked with the working solution of pesticides at

Table 2
SRM transitions and ESI-MS/MS optimized parameters.

Compound	1st transition (quantification)	DP[a]	CE[b]	CXP[c]	2nd transition (confirmation)	DP	CE	CXP
Acetochlor	270/224	25	20	5	270/148	25	20	5
Alachlor	270/162	25	30	4	270/238	25	30	4
Atrazine	216/174	25	25	4	216/104	25	25	4
Azoxystrobin	404/372	46	21	14	404/329	46	41	10
Carbaryl	202/145	41	15	6	202/127	41	39	6
Carbendazim	192/160	26	27	4	192/105	26	53	6
Carbofuran	222/165	41	17	6	222/123	41	31	6
3-Hydroxycarbofuran	238/163	46	19	6	238/107	46	43	4
Chlorfenvinphos	359/99	51	45	6	359/170	51	45	6
Chlorpyriphos	350/198	51	51	4	350/97	51	51	4
Chlortoluron	213/72	25	35	4	213/46	25	35	4
DCPMU	219/127	35	40	4	219/162	35	40	4
DCPU	205/127	30	40	4	205/162	30	40	4
DEA	188/146	30	25	3	188/104	30	25	3
DET	202/146	30	25	4	202/104	30	25	4
DIA	174/104	30	35	3	174/132	30	35	3
Dimethoate	230/199	41	13	6	230/125	41	29	6
Dimetomorph	388/301	26	29	10	388/165	26	41	6
Diuron	233/72	25	40	3	233/46	25	40	3
Hexazinon	253/171	21	21	6	253/71	21	49	4
IPPMU	193/94	30	30	4	193/151	30	30	4
IPPU	179/137	30	30	4	179/94	30	30	4
Irgarol	254/198	30	30	4	254/91	30	30	4
Isoproturon	207/72	30	35	4	207/165	30	35	4
Linuron	249/160	30	30	4	249/182	30	30	4
Metazachlor	278/134	25	30	4	278/210	25	30	4
Methomyl	163/88	21	13	4	163/106	21	13	4
Metolachlor	284/252	25	30	4	270/176	25	30	4
Metoxuron	229/72	30	40	3	229/46	30	40	3
Pyrimicarb	239/72	21	35	4	239/182	21	21	6
Simazine	202/132	30	30	4	202/124	30	30	4
Terbuthylazine	130/174	30	25	4	230/146	30	25	4
Thiodicarb	355/88	21	27	4	355/73	21	89	2
Atrazine-*d5*	221/179	25	25	4				
Carbaryl-*d3*	205/145	14	15	6				
Carbofuran-*d3*	225/123	41	31	6				
Chlorpyriphos-*d10*	360/107	51	51	4				
DEA-*d6*	194/147	30	25	3				
Diuron-*d6*	239/78	25	40	3				
Methomyl-*d3*	166/88	11	15	6				
Metolachlore-*d6*	290/258	25	30	4				
Monuron-*d6*	205/78	30	30	4				
Prometryn-*d6*	248/159	25	25	4				
Simazine-*d5*	207/137	30	30	4				

[a] DP: declustering potential.
[b] CE: collision energy.
[c] CXP: cell exit potential.

six levels: 1, 2, 5, 10, 25 and 50 $\mu g\,L^{-1}$. These calibration curves were analyzed with the LC–ESI-MS/MS method. Calibration curves were determined for each matrix, and the slopes of the four fortified matrixes were compared to the slope obtained with the standards in UPW.

Eight internal standards were used to minimize the effect of matrix components. These internal standards where chosen according to the retention time of the molecules and to their structure when it is possible to combine the two conditions. Diuron-*d6* to correct chlortoluron, DCPMU, DCPU, diuron, IPPMU, IPPU, isoproturon, linuron, metoxuron and the surrogate monuron-*d6*. Metolachlor-*d6* to correct acetochlor, alachlor, metazachlor and metolachlor. Atrazine-*d5* to correct atrazine, DET, hexazinon, irgarol, simazine, terbuthylazine azoxystrobin, dimetomorph and the two surrogates: prometryn-*d6* and simazine-*d5*. Carbaryl-*d3* to correct carbaryl, chlorfenvinphos, pyrimicarb and thiodicarb. Methomyl-*d3* to correct carbendazim, methomyl and dimethoate. Carbofuran-*d3* to correct carbofuran and carbofuran-3-hydroxy. DEA-*d6* to correct DEA and DIA. Chlopyriphos-*d10* to correct chlorpyriphos.

In the case of POCIS extracts, evaluation of matrix effects were investigated with the response of four internal standards (i.e. DEA-*d6*, diuron-*d6*, metolachlor-*d6* and atrazine-*d5*) according to two dilution levels (i.e. 2- and 10-fold). POCIS extracts were obtained from 14-day exposure in the *Ruiné* stream (south-west of France).

4. Results and discussion

4.1. Optimization of solid phase extraction conditions

4.1.1. SPE cartridge type selection and drying test

A comparison between different configurations and sorbent masses was performed: Oasis HLB with either 60 mg, 150 mg or 500 mg of sorbent, and Chromabond HR-X with 60 mg of sorbent. 50 mL tap water samples were spiked with the 33 analytes of interest at a concentration of 0.2 $\mu g\,L^{-1}$ for each compound. The recoveries ranged between 22.8 and 125.6%, and for a given elution volume (2 fractions of 3 mL) and solvent composition (MeOH for the first one and MeOH/ethyl acetate, 75/25 (v/v), for the second), the recoveries slightly decreased with the amount of sorbent (Fig. 1a). Chlorpyriphos showed the lowest recovery due to a high retention on the reversed phases. Rodrigues et al. obtained similar results with recoveries ranging between 6.8 and 76.5% with different cartridges: Oasis HLB (Waters), LiChrolut EN (Merck), and C18

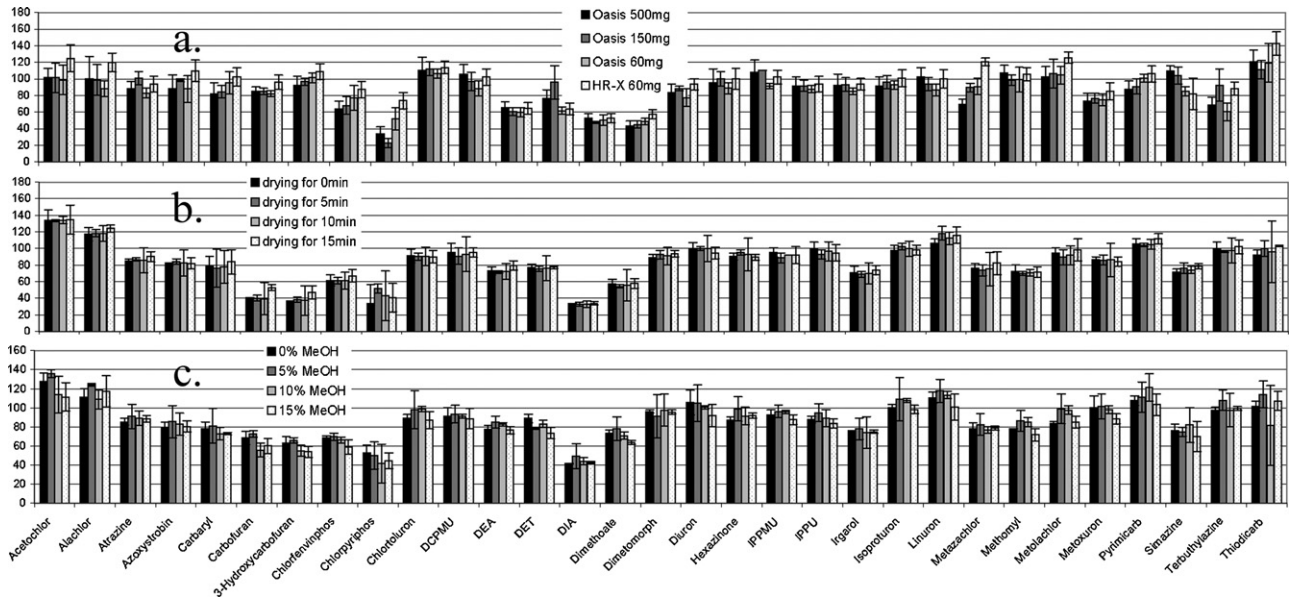

Fig. 1. (a) Recovery evolution with different Oasis HLB cartridges (60, 150 and 500 mg of adsorbent) and Chromabond HR-X 60 mg with internal calibration ($n = 5$), (b) recovery evolution with different drying times on Chromabond HR-X 60 mg with internal calibration ($n = 3$), (c) recovery evolution with different percentages of methanol in the washing step with the use of 60 mg Chromabond HR-X cartridges (with internal calibration, $n = 3$). Tap water was spiked at a concentration of 0.2 $\mu g\,L^{-1}$ for each compound for all of the three studies.

efc, C8 and HR-p (Macherey-Nagel) [24]. We obtained acceptable recoveries for this molecule (about 75%) with the 60 mg cartridges and without increasing the elution volume. With the Oasis 500 mg and 150 mg cartridges we only obtained recoveries of 33.6 and 22.8%, respectively. For other compounds, recoveries were acceptable (higher than 50%) except for carbendazim (data not show in Fig. 1) which showed recoveries lower than 15% when the spiked sample was prepared using tap water. Makihata et al. related the same problems with tap water for six molecules including carbendazim [25]. They explain their low recoveries by a degradation phenomenon due to residual chlorine in the water they used. We performed further experiments (data not shown) showing that this suppression effect is partially resolved by the use of Oasis MAX cartridges. These results indicate that the poor recovery for carbendazim was rather due to the elution step than a degradation process. However, using Oasis MAX cartridges gave poor recoveries for many carbamate pesticides and other molecules in various aqueous matrixes. Therefore, extraction with Oasis HLB or Chromabond HR-X sorbents was preferred. Regarding recoveries, Chromabond HR-X cartridge gave the best values, so this cartridge was preferred for the other experiments.

The influence of the drying step was investigated with Chromabond HR-X. Different drying times ranging from 0 to 15 min were tested, after the conditioning step with MeOH and UPW, and before the sample loading. Bouvier et al. have shown that sorbents based on polystyrene-divinylbenzene copolymer are particularly affected by a drying step [26]. The Chromabond HR-X cartridges contain a hydrophobic polystyrene-divinylbenzene copolymer and in our case, the impact of the drying time did not seem to be significant (Fig. 1b). This step can even be omitted as shown by further experiments for the set of pesticides selected. For instance, Bouvier et al. dried the sorbent between the two fractions of the conditioning step (i.e. just after methanol and before the ultrapure water). Methanol used for the conditioning is more volatile than water and it was probably eliminated during the drying step, decreasing sorbent wettability. Investigated recent patent mentioned the drying effect on SPE sorbents [27]. They compared a new copolymeric sorbent based on N-vinylimidazole/divinylbenzene (NVI–DVB) equivalent to the Oasis HLB (based on N-vinylpyrrolidone) with other sorbents like C18-RP or styrene-divinylbenzene copolymer and demonstrated that NVI–DVB was more resistant than the others and gave better recoveries. The drying effects were evaluated with similar conditions in this patent and in our work. The range of $\log K_{ow}$ tested by the inventors covered from −0.23 to 3 and corresponded to very polar and polar molecules, whereas our range of $\log K_{ow}$ corresponded to polar and non-polar molecules ($\log K_{ow}$: 0.6 to 4.96). However, the recoveries were not affected by the drying step for the most polar of our analytes with $\log K_{ow} < 1$ (i.e. methomyl, DIA, dimethoate and carbofuran-3-hydroxy). Overall, these results suggest that for our set of pesticides, a simple polystyrene-divinylbenzene copolymer gives satisfactory and robust recoveries.

4.1.2. Sample loading volume and clean-up step

The influence of the sample loading volume was investigated. The loading volume was investigated from 20 to 200 mL of water fortified with 0.4 $\mu g\,L^{-1}$. Poor limits of quantification (LOQs) were obtained with the lowest volume, whereas higher matrix effects were observed with volumes of 100 or 200 mL (data not shown). The best compromise between LOQs and matrix interferences was obtained with 50 mL. In addition, relatively fast extraction was achieved with this volume. Methanol addition to UPW during the washing step was also considered for removing interfering compounds and improving the accuracy. Whatever the percentage of methanol added, recoveries were not strongly impacted and a value of 5% MeOH in UPW was chosen (Fig. 1c). In conclusion, the following parameters were selected for the extraction step: Chromabond HR-X cartridges with 60 mg of adsorbent, 50 mL of sample and a washing step with 3 mL of UPW with 5% (v/v) of methanol.

4.1.3. Recoveries and LOQs

After the optimization of the SPE conditions, recoveries were calculated. Recoveries were determined over a long period (January and July 2009). 50 mL of spiked water was extracted on SPE cartridges at two levels: 0.04 $\mu g\,L^{-1}$ and 0.2 $\mu g\,L^{-1}$. Both mineral water (Evian) and river water from the *Ruiné* stream were used. Water samples were previously extracted to quantify the initial traces of the molecules used for the spike. These values were deducted

Table 3
Average recoveries ($n = 12$) of 33 pesticides after the SPE of 50 mL of two different waters spiked with two different waters with two different levels (0.04 μg L^{-1} and 0.2 μg L^{-1}), the relative standard deviations (RSD %) in brackets, and limits of quantification (LOQs).

	Mineral water (Evian)			Natural water (Ruiné)			LOQs (ng L^{-1})
	Average 0.04 ppb (RSD %)	Average 0.2 ppb (RSD %)	Average of the two spiking levels	Average 0.04 ppb (RSD %)	Average 0.2 ppb (RSD %)	Average of the two spiking levels	
Acetochlor	97 (23)	88 (21)	92 (22)	108 (27)	97 (19)	102 (24)	20
Alachlor	107 (26)	85 (21)	96 (26)	109 (39)	89 (15)	99 (31)	40
Atrazine	96 (17)	94 (15)	95 (16)	107 (33)	97 (11)	102 (25)	20
Azoxystrobin	87 (27)	88 (14)	88 (21)	77 (18)	74 (7)	76 (13)	20
Carbaryl	–	97 (26)	–	–	111 (18)	–	100
Carbendazim	77 (32)	86 (12)	82 (24)	55 (29)	57 (12)	56 (21)	20
Carbofuran	58 (21)	61 (20)	60 (20)	83 (22)	82 (11)	82 (17)	20
3-Hydroxycarbofuran	52 (16)	53 (21)	52 (19)	49 (28)	53 (11)	51 (21)	40
Chlorfenvinphos	113 (36)	170 (60)	148 (57)	129 (36)	135 (42)	132 (38)	20
Chlorpyriphos	84 (25)	63 (13)	73 (22)	68 (34)	65 (17)	66 (26)	40
Chlortoluron	106 (19)	103 (26)	105 (23)	106 (12)	95 (10)	100 (12)	20
DCPMU	91 (14)	90 (16)	91 (14)	108 (24)	96 (11)	102 (19)	40
DCPU	99 (41)	96 (21)	97 (31)	100 (34)	100 (19)	100 (27)	100
DEA	86 (13)	83 (11)	85 (12)	112 (54)	91 (14)	100 (38)	20
DET	98 (18)	98 (19)	98 (18)	107 (20)	104 (18)	105 (19)	20
DIA	94 (15)	87 (15)	91 (15)	97 (43)	82 (18)	89 (32)	20
Dimethoate	74 (13)	71 (7)	73 (10)	72 (11)	68 (5)	70 (9)	20
Dimetomorph	96 (23)	91 (15)	94 (19)	94 (25)	80 (11)	87 (20)	20
Diuron	105 (18)	99 (19)	102 (18)	108 (14)	102 (19)	105 (16)	20
Hexazinon	105 (30)	117 (21)	111 (26)	84 (23)	86 (7)	85 (17)	20
IPPMU	104 (21)	97 (9)	100 (16)	95 (13)	87 (7)	91 (11)	20
IPPU	87 (31)	88 (8)	88 (22)	89 (13)	86 (7)	87 (10)	20
Irgarol	89 (19)	79 (13)	84 (17)	80 (8)	75 (8)	77 (8)	20
Isoproturon	101 (19)	92 (7)	97 (14)	100 (12)	94 (9)	97 (11)	20
Linuron	98 (15)	89 (12)	93 (14)	114 (19)	105 (21)	109 (20)	20
Metazachlor	95 (21)	92 (16)	93 (18)	72 (18)	64 (19)	68 (19)	20
Methomyl	98 (16)	94 (19)	96 (17)	97 (12)	93 (15)	95 (13)	20
Metolachlor	96 (24)	110 (59)	104 (47)	88 (16)	87 (26)	87 (23)	40
Metoxuron	93 (21)	87 (7)	90 (16)	82 (11)	76 (9)	79 (10)	20
Monuron-*d6*[a]	109 (22)	106 (11)	107 (17)	111 (41)	103 (8)	107 (29)	–
DIA-*d5*[b]	113 (27)	96 (17)	105 (24)	107 (23)	99 (16)	103 (20)	–
Prometryn-*d6*[a]	103 (24)	98 (13)	101 (19)	103 (42)	95 (13)	99 (31)	–
Pyrimicarb	95 (22)	102 (6)	99 (15)	111 (7)	108 (16)	110 (12)	20
Simazine	92 (19)	92 (16)	92 (17)	87 (26)	94 (15)	91 (21)	20
Simazine-*d5*[a]	101 (27)	97 (10)	99 (20)	103 (31)	94 (6)	98 (22)	–
Terbuthylazine	90 (15)	86 (14)	88 (14)	101 (16)	95 (14)	98 (15)	20
Thiodicarb	91 (17)	129 (18)	114 (26)	109 (22)	112 (13)	111 (18)	20

[a] Surrogates.
[b] Performance and reference compound.

to calculate the average recoveries. The recoveries were corrected with the matched internal standards. The surrogates were only used to control the SPE procedure. The recoveries of the surrogates were acceptable and no correction with the surrogate was performed. Recoveries were between 51 and 148% (Table 3). 32 molecules in mineral water and 28 in natural water showed recoveries between 80 and 120%. Poor results in Evian water were obtained for chlorfenvinphos and also 3-hydroxycarbofuran and maybe carbofuran. 3-Hydroxycarbofuran has probably unresolved matrix effects. The origin of the enhancement of chlorfenvinphos response was probably due to the difference between the intensity of the compound and the internal standard. Carbaryl-*d3* was initially the matching internal standard for chlorfenvinphos but its intensity was too low to correct this molecule. Consequently, we tested another internal standard (pirimicarb-*d6*), which seemed to give better results (recoveries of $107 \pm 9\%$ at level 0.04 μg L^{-1} ($n = 3$) and of $93 \pm 6\%$ at level 0.2 μg L^{-1} ($n = 3$)). Natural water showed more fluctuating results but all recoveries were acceptable. Only carbofuran-3-hydroxy and carbendazim gave results close to 50% probably due to unresolved matrix effects during the SPE procedure. For chlorpyriphos, the recovery was under 20% when the results were interpreted without any internal standard. This phenomenon is not due to a matrix effect since further experiments revealed that losses occurred during the SPE procedure, more precisely after eluent elimination. It turns out that chlorpyriphos was affected by evaporation (Henry's Law Constant = 2.93×10^{-6} whereas the other molecules showed values between 7.3×10^{-14} and 3.72×10^{-8}). This problem was overcome with the use of the corresponding deuterated compound as internal standard, and its addition before evaporation to dryness.

The LOQs were determined by the spiking of tap water at two levels (20 and 40 ng L^{-1}, $n = 10$) (Table 3). Only carbendazime LOQ was determined in a natural water sample because of SPE problems in tap water. 26 molecules were validated with a LOQ of 20 ng L^{-1} and 5 with a LOQ of 40 ng L^{-1}. Two of them, carbaryl and DCPU, were not validated at 40 ng L^{-1} and presented a LOQ of 100 ng L^{-1}.

4.2. Matrix effects and interferences

The impact of matrix effects on quantification can be particularly important since it results in serious inaccuracies in pollutant analysis. There are several ways of overcoming or reducing these matrix effects, the most commonly used being: standard additions [28], matrix matched calibration [29], internal standard corrections [28,30], echo-peak technique [31] and sample extract dilutions [30,32]. Some of these approaches are really time-consuming and cannot be used in routine analysis (e.g. standard additions and matrix matched calibration). In our work, the internal standard method was chosen for the SPE with isotopic labeled compounds. However, deuterated or other isotopic labeled compounds are not

Table 4
Comparison of resolution (R_s) and selectivity (α) with two Gemini-NX column lengths and for the three most polar analytes (methomyl, DIA and carbendazim) and for six selected analytes from a separation domain of 2 min in the middle of the chromatogram (Fig. 2).

Compounds	Selectivity α		Resolution R_s		Retention times (min)	
	α 50 mm	α 100 mm	R_s 50 mm	R_s 100 mm	50 mm	100 mm
Methomyl/DIA	–	–	0.61	1.88	1.5/1.75	2.81/3.28
DIA/carbendazim	–	–	4.41	9.08	1.75/3.58	3.28/4.69
IPPMU/Thiodicarb	1.01	1.02	0.55	0.59	6.27/6.34	7.59/7.52
Thiodicarb/Chlortoluron	1.00	1.04	0.07	1.73	6.34/6.35	7.52/7.76
Chlortoluron/Pyrimicarb	1.01	1.00	0.21	0.07	6.35/6.38	7.76/7.75
Pyrimicarb/Atrazine	1.04	1.07	1.26	2.20	6.38/6.57	7.75/8.08
Atrazine/Isoproturon	1.08	1.07	2.51	2.45	6.57/6.96	8.08/8.45

commercially available for some classes of analytes (e.g. dicarboximides, morpholines and strobilurines) or not appropriate (i.e. chlorpyriphos-*d10* and carbaryl-*d3* were less suitable than pirimicarb-*d6* for the quantification of chlorfenvinphos). Generally, internal standard calibration is sufficient to overcome matrix effects with a SPE procedure of water samples [33,34]. However, the choice of these internal standards can be difficult when the method includes a large number of chemicals [35]. Even if the retention time is a good indicator to choose the matching internal standard, the molecule structure has to be taken into account and can be sometimes more relevant [36]. For a multiresidue method it is necessary to make a compromise and test different combinations.

4.2.1. Influence of analytical column length on resolution and matrix effects

The use of shorter analytical columns is increasingly approved by a large majority of analytical chemists. They lead to shorter analysis times allowing more samples to be processed by laboratories. However, reducing the separation time may result in a loss of resolution and in the co-elution of a larger number of compounds including matrix interfering components. In order to slow down the elution of these molecules we tested different possibilities such as changing the percentage or the composition of the eluents (e.g. methanol instead of acetonitrile) and the flow rate but convincing results were lacking. Another way was to work with a narrow bore and a longer column to improve the efficiency with a short analysis time and low flow rate. Two different lengths were tested (50 × 2 and 100 × 2 mm) for the Gemini-NX C18 3 μm, 110 Å column, with the same analytical gradient. Obviously, the retention time increased for all the analytes (Table 4), especially for the first eluting peaks. The selectivity and the resolution were also improved with the longer column for most of the compounds (Fig. 2 and Table 4). Selectivity calculations (α) showed that the separation was better with the 100 mm column. The resolutions (R_s) were also better with the 100 mm column except for the couple chlortoluron/pyrimicarb (the two analytes were not separated whatever the column length). For the 50 mm column three couples of molecules were not separated whereas for the 100 mm column there was only two couple of molecules that remained unresolved. Finally, for the 50 mm column only two couples were clearly separated whereas for the 100 mm column, there are five couples of molecules with an acceptable or even good separation.

Matrix effects were evaluated with three types of freshwater: river water from *Boutonne* river, pond water and tap water. To obtain matrix extracts, SPE was done with 50 mL of each water. Then, calibration curves were drawn for each matrix with 6 levels (1, 2, 5, 10, 25 and 50 μg L^{-1}) and they were compared to the same calibration curve in ultrapure water. Matrix effects (MEs) were determined by the ratios of the two slopes:

$$ME = \frac{a_{upw}}{a_{matrix}} \quad (5)$$

where a_{upw} and a_{matrix} are the slopes of the calibration curves in UPW and the matrix of interest, respectively.

A value higher than 1 suggests signal enhancement whereas a value lower than 1 indicates an ion-suppression effect. Results have shown differences ranging from approximately 0 to 100% between these two column lengths and also the dependence on the type of water (Fig. 3). Obviously, pond water contains higher amounts of interfering compounds as indicated by dissolved organic carbon (DOC) analysis: approximately 40 mg L^{-1} for pond water, 4 mg L^{-1} for river water, 2 mg L^{-1} for tap water and 0.7 mg L^{-1} for mineral water [37]. Tap water gave also some suppression effects and river water seems to be the matrix with the lowest interferences. Except for carbendazim, the use of a shorter column results in stronger signal suppression (most of the analytes) or signal enhancement (e.g. carbaryl). A very short column leads to faster analyses with a slight loss of selectivity and resolution. However, our observations indicate that the analyte responses are less affected by matrix effects with a column only twice as long, which enables HPLC analysis time to be kept relatively short.

4.2.2. Matrix effects with the SPE protocol

After optimization of the SPE protocol and the choice of the analytical column length, we studied the matrix effects for the 33 chemicals of interest with four different waters. The matrix effects were evaluated following application of Eq. (5) and an overview of the results is given in Table 5 (external and internal standards). For the mineral water, matrix effects were negligible across the whole range of molecules. For a few molecules, matrix effects were slightly higher; highest for carbendazim, diuron, metazachlor and terbuthylazine; since there was a low suppression effect for a few molecules. In this case, internal standard calibration did not lead to a real improvement except for carbendazim and diuron. Two molecules, DCPU and thiodicarb, exhibited a signal enhancement effect. Concerning DCPU, this result was due to a poor detection with ESI-MS/MS. For thiodicarb, the cause of the enhancement is certainly the internal standard used for the correction of the matrix effect, i.e. carbaryl-*d3*. We have seen that carbaryl had a very low sensitivity and the response of deuterated carbaryl was even lower. Thiodicarb had a better response than its internal standard (carbaryl-*d3*) in mass spectrometry. Difference was too high for a satisfactory correction. As for chlorfenvinphos, we have tested pirimicarb-*d6* as a new internal standard and it was used for the further applications. For the pond water, two molecules suffered from high ion suppression effects (carbendazim and metoxuron) and one had a signal enhancement effect (DCPU). Internal calibration was not sufficient to overcome these signal suppressions. Lastly, tap water showed some suppression effects for two molecules, carbendazim and metazachlor. Internal standard calibration overcame the problem for carbendazim but not for metazachlor.

4.2.3. Matrix effects with the POCIS extracts

In order to calculate the TWACs the POCIS must be calibrated for all compounds to be monitored and for the sampling

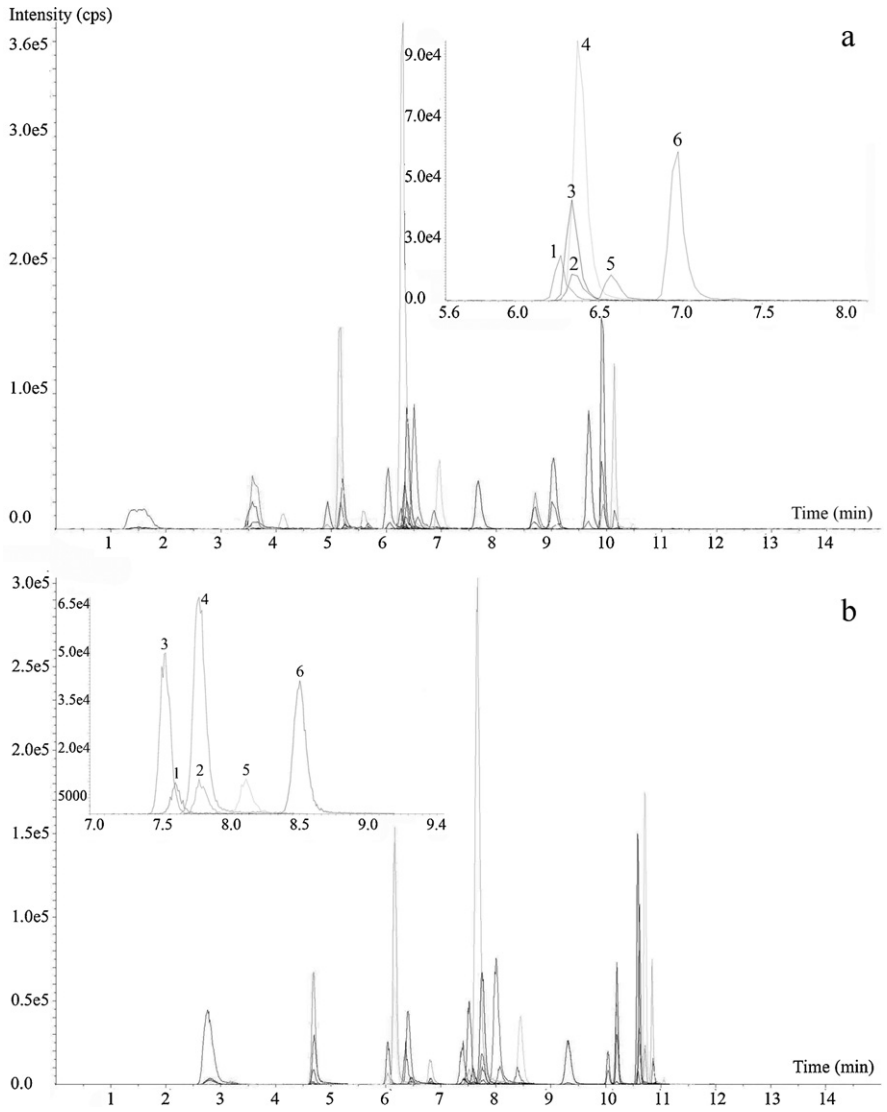

Fig. 2. Separation of the 33 pesticides with two Gemini-NX column lengths: 50 mm (a) and 100 mm (b). Peak numbers: (1) IPPMU, (2) chlortoluron, (3) thiodicarb, (4) pyrimicarb, (5) atrazine and (6) isoproturon.

rate ($R_{s\,cal}$) calculated. A previous work provided the R_s values for 17 polar pesticides [22]. In the present work, this calibration was performed for 33 molecules (Table 6). A good agreement was obtained for the R_s values of the 11 pesticides in common between this study and that of Mazzella et al. [22].

POCIS devices concentrate large volumes of water when they are exposed for a long period. For example, 14 days of exposure

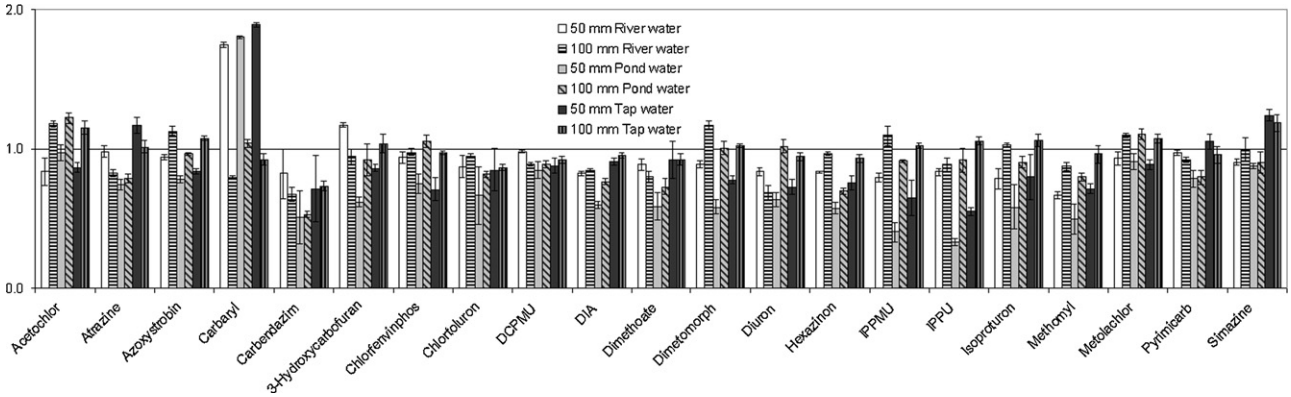

Fig. 3. Matrix effects on selected analytes with different waters (river water from *Boutonne*, a *Charente* affluent in south west part of France, tap water and pond water) and two analytical column length Gemini-NX (50 mm and 100 mm).

Table 5
Matrix effects with different waters (mineral water (*Evian*), tap water, river water from Boutonne and pond water) with Chromabond HR-X 60 μm SPE cartridges and Gemini-NX C18 3 μm, 110 Å, 100 × 2 mm analytical column.

	Evian (RSD %)	Evian IS[a] correction (RSD %)	Tap water (RSD %)	Tap water IS[a] correction (RSD %)	Boutonne (RSD %)	Boutonne IS[a] correction (RSD %)	Pond water (RSD %)	Pond water IS[a] correction (RSD %)
Acetochlor	1.02 (6)	1.10 (7)	1.15 (4)	1.04 (6)	1.18 (2)	1.10 (3)	1.22 (3)	1.14 (7)
Alachlor	0.97 (2)	1.05 (5)	1.19 (4)	1.07 (6)	1.10 (3)	1.02 (4)	1.05 (3)	0.96 (7)
Atrazine	0.98 (1)	1.13 (2)	1.01 (2)	0.99 (4)	0.83 (4)	0.93 (5)	0.79 (1)	0.89 (3)
Azoxystrobin	1.13 (1)	1.31 (2)	1.08 (4)	1.05 (6)	1.13 (1)	1.27 (2)	0.96 (3)	1.09 (4)
Carbaryl	0.82 (8)	0.79 (9)	0.92 (4)	0.87 (6)	0.79 (6)	0.85 (7)	1.04 (2)	0.90 (4)
Carbendazim	0.92 (2)	0.96 (2)	0.73 (9)	0.83 (9)	0.67 (8)	0.82 (5)	0.53 (22)	0.65 (20)
Carbofuran	1.05 (3)	1.09 (4)	1.17 (1)	1.05 (3)	1.06 (3)	1.04 (3)	1.23 (4)	1.09 (6)
3-Hydroxycarbofuran	0.98 (1)	1.00 (2)	1.04 (2)	0.93 (5)	0.95 (1)	0.94 (4)	0.92 (2)	0.81 (5)
Chlorfenvinphos	1.10 (1)	1.05 (2)	0.97 (2)	0.92 (4)	0.98 (1)	1.04 (2)	1.06 (2)	0.92 (6)
Chlorpyriphos	0.33 (62)	1.12 (5)	0.15 (280)	1.11 (6)	0.18 (437)	0.91 (9)	0.44 (31)	1.02 (6)
Chlortoluron	0.98 (4)	0.97 (1)	0.87 (3)	0.77 (6)	0.95 (2)	1.03 (2)	0.82 (3)	0.73 (5)
DCPMU	0.93 (6)	0.92 (3)	0.92 (4)	0.82 (6)	0.89 (4)	0.97 (5)	0.89 (7)	0.80 (12)
DCPU	1.07 (8)	1.09 (8)	1.19 (7)	1.06 (8)	1.32 (4)	1.43 (3)	1.51 (3)	1.33 (3)
DEA	0.93 (2)	0.95 (3)	0.94 (3)	0.96 (4)	0.89 (4)	1.00 (3)	0.82 (3)	0.86 (7)
DET	0.95 (2)	1.09 (1)	1.07 (4)	1.05 (6)	0.89 (3)	1.00 (4)	0.82 (2)	0.93 (4)
DIA	0.96(2)	0.99 (2)	0.95 (1)	1.08 (1)	0.85 (4)	1.02 (3)	0.77 (7)	0.94 (7)
Dimethoate	0.98 (1)	1.01 (0)	0.92 (3)	1.05 (3)	0.80 (7)	0.97 (5)	0.73 (6)	0.89 (7)
Dimetomorph	1.33 (1)	1.54 (1)	1.02 (3)	1.00 (5)	1.17 (2)	1.32 (2)	1.01 (2)	1.14 (4)
Diuron	0.94 (2)	0.95 (2)	0.94 (2)	0.84 (4)	0.69 (11)	0.88 (10)	1.02 (1)	0.91 (2)
Hexazinon	1.07 (2)	1.23 (3)	0.93 (3)	0.90 (7)	0.97 (5)	1.09 (6)	0.70 (13)	0.79 (13)
IPPMU	1.06 (4)	1.04 (1)	1.02 (4)	0.91 (5)	1.09 (1)	1.18 (1)	0.91 (4)	0.81 (3)
IPPU	0.98 (3)	0.99 (4)	1.06 (6)	0.94 (6)	0.89 (3)	0.96 (2)	0.92 (3)	0.82 (1)
Irgarol	1.02 (1)	1.17 (2)	0.99 (4)	0.97 (7)	0.89 (4)	1.01 (5)	0.88 (5)	1.00 (6)
Isoproturon	1.06 (2)	1.07 (3)	1.06 (3)	0.95 (3)	1.03 (2)	1.12 (1)	0.90 (4)	0.80 (2)
Linuron	1.00 (1)	1.01 (3)	1.05 (2)	0.93 (4)	1.05 (1)	1.13 (1)	1.08 (2)	0.96 (5)
Metazachlor	0.97 (4)	1.05 (7)	0.78 (8)	0.70 (13)	0.74 (3)	0.69 (5)	0.77 (7)	0.71 (17)
Methomyl	0.91 (2)	0.94 (2)	0.96 (6)	1.10 (6)	0.88 (9)	1.07 (6)	0.80 (10)	0.98 (9)
Metolachlor	0.99 (3)	1.05 (1)	1.07 (2)	0.96 (3)	1.10 (2)	1.02 (3)	1.11 (3)	1.02 (6
Metoxuron	1.01 (2)	1.02 (4)	0.85 (5)	0.76 (6)	0.81 (4)	0.87 (2)	0.63 (18)	0.55 (14)
Pyrimicarb	0.84 (3)	0.80 (4)	0.96 (1)	0.90 (3)	0.92 (2)	0.98 (3)	0.80 (1)	0.69 (7)
Simazine	1.14 (2)	1.31 (3)	1.19 (3)	1.16 (5)	1.00 (3)	1.13 (4)	0.90 (4)	1.02 (5)
Terbuthylazine	1.06 (1)	1.22 (1)	1.12 (3)	1.09 (5)	0.76 (15)	0.73 (33)	0.96 (3)	1.09 (4)
Thiodicarb	1.34 (2)	1.23 (3)	1.11 (6)	1.02 (7)	1.52 (3)	1.60 (3)	1.33 (6)	1.08 (6)

[a] IS: internal standardization.

corresponds to a pre-concentration of about 1–3.5 L of water with a classical SPE approach. Previously, we observed some matrix effects with 50 mL of water extracted by SPE, thus we may expect similar phenomena with the POCIS extracts. In the early stages of our work, standard additions were performed (data not shown). As mentioned in a previous paper, it gave acceptable corrections of matrix effects with a standard deviation of ±20% [23]. However, this method is also very time-consuming and not applicable to routine analysis. Therefore, we diluted the POCIS extracts and investigated the influence of the dilution levels on matrix effects. The matrix effects were evaluated with the relative response of the isotopic labeled compound used as internal standards. The no-effect value should be close to 1 (ratio between the internal standard response in UPW and POCIS extracts).

For non-diluted POCIS extract, some analytes presented concentrations exceeding the range of the calibration curve. Then, post-elution dilution was compulsory and we decided to dilute POCIS extracts 2-fold. Results showed that the responses of the internal standards differed in calibration solutions and samples (Fig. 4a). DEA-*d6* underwent a high suppression effect (reaching 50%), whereas diuron-*d6* and metolachore-*d6* were enhanced. Dilution by a factor of ten was further tested with the POCIS extracts. Suppression or enhancement of the signals of internal standards with these dilutions ranged from 0.8 to 1.2, which were acceptable values. A long-term study was carried out with a 10-fold dilution of several POCIS exposed from April to November 2009 in river water (14-day exposure periods). The results (Fig. 4b) indicate that this dilution led to a reduction of the impact of matrix interfering components. Some POCIS extracts (April and June I) gave results higher than 1.2 with a maximum of 1.3 for diuron-*d6* and atrazine-*d5* in June I and two POCIS extracts (August I and October II) gave a result of under 0.8 with a minimum of 0.75 for atrazine-*d5* in October

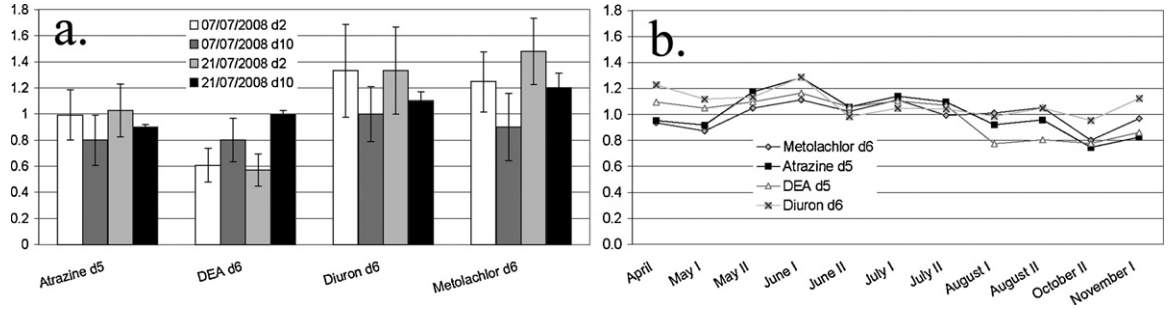

Fig. 4. (a) POCIS exposed to river water (Ruiné) for 14 days in July 2008 (7th to 21st July and 21st July to 4th August) and influence of dilution (2-fold (d2) and 10-fold (d10)) on selected internal standard response. (b) Matrix effects on POCIS extracts with a 10-fold dilution during the 2009 campaign (April–November on Ruiné stream).

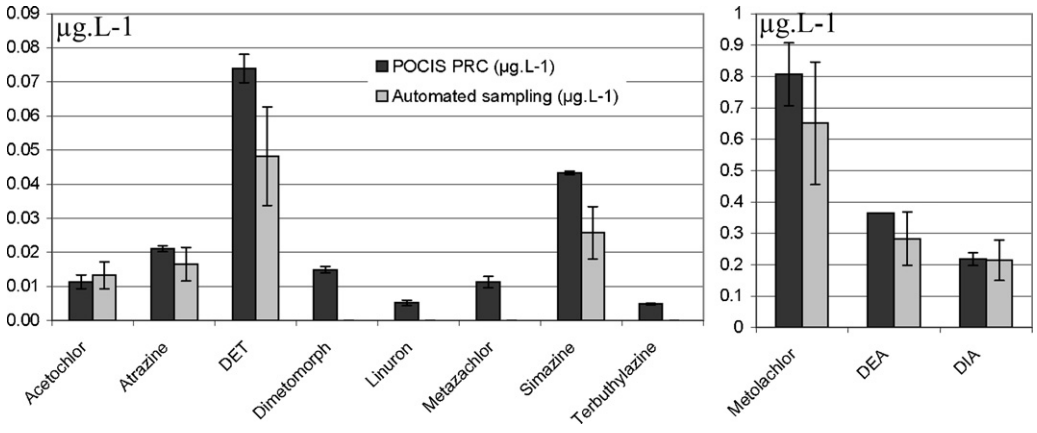

Fig. 5. Comparison between automated sampling with SPE (two week average concentrations) and passive sampling (POCIS exposure for 14 days: 25st May to 8th June) in Ruiné stream.

Table 6
Calibration of the POCIS for the 33 pesticides studied.

	$k_{u(cal)}$[a] ($L\,g^{-1}\,d^{-1}$)	R_s[b] ($mL\,d^{-1}$)	RSD (%)[c]	Linearity[d]
Acetochlor	1.206	241	14	0.98
Alachlor	1.026	205	2	0.96
Atrazine	1.138	228	18	0.98
Azoxystrobin	0.894	179	12	0.98
Carbaryl	1.217	243	19	0.97
Carbendazim[e]	N/A	N/A	N/A	N/A
Carbofuran	1.409	282	21	0.99
3-Hydroxycarbofuran	0.985	197	11	0.99
Chlorfenvinphos	1.391	278	11	0.92
Chlortoluron	0.826	165	21	0.99
Chlorpyriphos	0.626	125	6	0.97
DCPMU	0.920	184	17	0.98
DCPU	0.994	199	23	0.99
DEA	0.865	173	11	0.99
DET	1.065	213	20	0.99
DIA	0.882	176	4	0.96
Dimethoate	1.035	207	7	0.99
Dimetomorph	0.850	170	14	0.98
Diuron	0.993	199	19	0.99
Hexazinon	0.796	159	16	0.98
IPPMU	0.931	186	19	0.99
IPPU	0.923	185	15	0.98
Irgarol	1.188	238	15	0.99
Isoproturon	0.837	167	20	0.99
Linuron	1.019	204	18	0.97
Metazachlor	1.026	205	16	0.98
Methomyl	0.434	87	5	0.94
Metolachlor	0.912	182	21	0.98
Metoxuron	0.881	176	17	0.99
Pyrimicarb	0.906	181	18	0.98
Simazine	0.994	199	19	0.99
Terbuthylazine	1.192	238	15	0.98
Thiodicarb	0.840	168	11	0.97

[a] k_u: accumulation kinetic constant.
[b] R_s: sampling rate.
[c] Relative standard deviation of k_u and R_s.
[d] Correlation coefficient (linear regression).
[e] Carbendazime was not quantified with neither POCIS or SPE since the calibration was performed in tap water.

II. Nevertheless, these suppression and enhancement phenomena were acceptable (−25/+30% of relative deviation) and covered less than 20% of the sampling period.

4.3. Comparison of the SPE limits of quantification with the POCIS concentration estimates

We compared automated sampling (associated with SPE method using 50 mL of water) and passive sampling (triplicate of POCIS exposed for 14 days: 25th May to 8th June) in the Ruiné stream (Fig. 5). All the POCIS extracts were diluted by ten and the estimated time-weighted average concentrations (TWACs) were determined with Eq. (1) and the EAF correction [23]. In one hand, estimates of TWACs were similar with POCIS and with automated sampling. In the other hand, with automated sampling we detected only seven chemicals (acetochlor, atrazine, DEA, DET, DIA, metolachlor and simazine) whereas with POCIS four others were detected and quantified (dimetomorph, linuron, metazachlor and terbuthylazine). Dimetomorph was quantified at 14.8 ng L^{-1}, linuron at 5.1 ng L^{-1}, metazachlor at 11.3 ng L^{-1} and terbuthylazine at 4.8 ng L^{-1}. The concentration capacity of the POCIS is clearly advantageous because, even with a 10-fold dilution, a higher number of molecules can be detected even though they occur at ultra-trace levels.

In addition to the high pre-concentration of analytes and the TWAC estimates, the passive sampling approach reduced laboratory manipulations and the use of solvents since the extraction step was performed directly *in situ*. Furthermore, the development of a POCIS-LC–ESI-MS/MS method requires the optimization of a limited number of parameters with respect to SPE methods whereas, the development of a SPE-LC–ESI-MS/MS method required the optimization of the SPE step with a difficult compromise between acceptable recoveries (i.e. acceptable LOQs) and low matrix effects. This compromise is even more difficult when the molecules to be detected present a wide range of polarities and structures (i.e. multiresidue method) and involves precise selection of matching internal standards. In comparison, for the POCIS, when sampling rates are available, only few adjustments (i.e. dilution of POCIS extracts and internal standard calibration) were necessary to obtain both acceptable accuracy and very low LOQs.

Acknowledgements

The authors would like to thank Sylvia Moreira and Gwilherm Jan for their technical support, Brigitte Méchin and Brigitte Delest for the laboratory analysis, AEAG (Agence de l'Eau Adour-Garonne) and ONEMA (Aquaref) for their financial support and Peter Winterton for revising the English-language version of the manuscript. Sophie Lissalde gratefully acknowledges the "Conseil Régional de Poitou Charentes" for the partial funding of her PhD.

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