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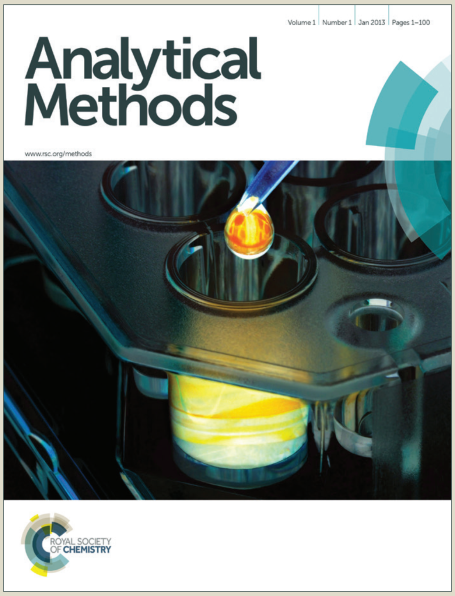

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EXPERIMENTAL DESIGN APPROACH TO THE OPTIMISATION OF PESTICIDES EXTRACTION FROM WATER

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Abstract

In the last few decades, pesticides have been used increasingly throughout the world. Nowadays, contamination of the aquatic systems by pesticides has become a global problem. Due to their stability, mobility and long-term effects on living organisms, pesticides are among the most dangerous pollutants that can be monitored in the environment, and the determination of accurate contamination levels also constitutes a crucial step in environmental research. However, in case of quantitative analyses, extraction of targeted analytes can turn out to be difficult since these compounds are often present below the detection limits. Consequently, the accuracy of environmental analyses mainly depends on the efficiency and the robustness of the extraction-preconcentration step. In this work, a solid-phase extraction (SPE) procedure using hydrophilic modified styrene-based polymer (HLB) cartridges was optimized for the extraction of organonitrogen and organochlorine pesticides in water. An experimental design was carried out for modeling SPE optimal extraction conditions of thirty four pesticides. The five parameters studied were flow rate, pH, elution speed, ionic strength of sample and the nature of the eluting solvent. Extracts were analyzed using gas chromatography equipped with mass spectrometer (GC-MS). The optimal extraction conditions selected for flow rate, pH, elution speed, ionic strength and nature of eluting solvent were respectively 2 - 4 mL.min^{-1}, pH = 6, 0.5 mL.min^{-1}, 100 g.L^{-1} of NaCl and ethyl acetate/methanol (1/1 *v/v*). The analytical procedure was validated for fifteen pesticides including thirteen organonitrogens and two organochlorines.

Keywords: Multi-residue analysis, organonitrogen pesticides, organochlorine pesticides, solid-phase extraction, GC-MS, experimental design

1. Introduction

Pesticides play a key role in world food production and have been widely used during these last decades. They can easily reach aquatic ecosystems by direct application, spray drift, aerial spraying, erosion, runoff from factories and sewage. It is commonly said that more than 95% of pesticides used in agriculture are dispersed in the environment, in air, water and sediment[1]. They can be now detected in surface waters, ground waters and even in glaciers[2,3]. However, problems related to continuous use of pesticides have increased worldwide until the contamination becomes a serious threat on both the aquatic ecosystem and the human health[4-8]. Various regulations have come into force concerning permissible levels of pesticide residues[9,10] and they led to develop new analytical techniques or to improve existing ones.

Extraction of pesticides from water can be carried out by various techniques such as liquid-liquid extraction (LLE)[11], matrix solid-phase dispersion (MSPD)[17], stir-bar sorptive extraction (SBSE)[18], solid-phase extraction (SPE)[12] technique or solid phase micro-extraction (SPME)[13]. Among these strategies, SPE has received an increasing attention because of its facility to implement, time saving, elimination of emulsions, and according to the fact that it considerably reduces the amount of solvent required[19,20]. In addition, better enrichment factors are usually obtained by SPE[21]. Nowadays, the solid-phase extraction has been proved to be a powerful method for sample preparation[10,22-25]. Furthermore, SPE presents a high potential for automation[9,26,27]. Indeed, even if the application of LLE in water have been widely accepted in standard methods, LLE procedure is time consuming and requires a large volume of solvent. Micro-LLE has been introduced in US Environmental Protection Agency (EPA) methods but such technique does not allow trace detection at low concentration level (0.1 µg.L^{-1}) as required for monitoring pesticides in drinking water in European countries[14-16].

Selection of the sorbent is an important aspect in using SPE technique[28]. Chemically bonded silica (e.g. C_{18} silica) and styrene/divinyl benzene (PS-DVB) copolymers are the most frequently sorbents used for extraction from water samples [9,24,29-32]. However, these sorbents often present low recoveries for polar compounds extraction. Moreover, C_{18} silica reveals to be unstable at extreme pH[33,34]. Porous graphitic carbon (PGC) has been used as sorbent due to its great adsorption capacity associated to its chemical, thermal and mechanical resistance. However, the use of such carbon sorbent can lead to excessive, or even irreversible retention[35,36]. Recently, new hydrophilic polymeric materials, obtained by copolymerizing monomers containing suitable functional groups or by introducing a functional group to the existing hydrophobic polymers, have been developed as SPE materials. Targeted benefits of these new functionalized polymeric sorbent are the improvement of the wetting characteristics, mass transfer and retention of polar or ionized compounds. The most common functionalized sorbent for large multi-residue extraction is the Oasis HLB (Waters®), which is a macroporous copolymer made from a balanced ratio of the lipophilic divinylbenzene and the hydrophilic *N*-vinylpyrrolidone, thus providing reversed-phase capability with a special hook for polar compounds[37-43].

SPE methods often involve investigation of many variables, which may affect the efficiency of extraction. By considering such a multi-criterion approach, an experimental design can be used to optimize important variables[10]. Optimization through experimental design often assumes factorial designs or non-linear models called response surface models, which require in this case three levels for each parameter [44].

In this work, Supel-Select HLB SPE (Sigma-Aldrich, USA) cartridges were employed through an experimental design model to optimize the extraction conditions of 34 pesticides in water. Five variables (sample flow rate, pH, ionic strength, nature of eluent and elution flow rate) were studied. Optimal conditions were then validated with natural water sample.

2. Materials and methods

2.1 Chemicals

Thirty four pesticides were firstly selected as target compounds (Table 1 - Figure 1). Pesticide standards were provided by Restek (Bellefonte, USA). Supel-Select HLB SPE cartridges (200 mg / 6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). Sorbent phase was characterized by particles size of 55-60 µm offering a pore size of 87 Å and a surface area of 400 $m^2.g^{-1}$. HPLC grade ethyl acetate (AcOEt), dichloromethane (DCM), methanol (MeOH), acetonitrile (ACN) and hexane were purchased from Dislab (Lens, France). Ultrapure water (Milli-Q) was produced by a Millipore apparatus (18.2 $MΩ.cm^{-1}$ resistivity). Sodium chloride (NaCl, 99.5 %) and analytical grade hydrochloric acid (HCl, 37 %) were purchased from Merck (Darmstadt Germany). Potassium hydroxide (KOH, 0.5 $mol.L^{-1}$ in methanol) was purchased from Panreac Quimica (Barcelona Spain). Pentachloronitrobenzene as internal standard with a purity of 94% was purchased from Sigma-Aldrich (Saint-Louis, USA). All pesticides solution standards were prepared in acetonitrile. GC-MS calibration was performed using eight calibration solutions ranging from 5 $µg.L^{-1}$ to 5 $mg.L^{-1}$ directly prepared from stock solutions.

Compounds	***Chemical class***	***Function***	***Log K_{OW}***	***RT (min)***	***LOD ($µg.L^{-1}$)***	***Qualifier Ions***
EPTC	Thiocarbamate	Herbicide	3.2	11.8	0.05	126;134
Butylate	Thiocarbamate	Herbicide	4.1	13.1	0.05	145;188
Vernolate	Thiocarbamate	Herbicide	3.8	14.0	0.05	127;147;160
Tebuthiuron	Urea	Herbicide	1.8	14.8	0.05	155;170
Etridiazole	Thiazole	Fungicide	2.6	15.8	0.05	183;211
Molinate	Thiocarbamate	Herbicide	3.2	17.8	0.05	126
Propachlor	Chloroacetamide	Herbicide	2.4	19.4	0.05	120;176
Cycloate	Thiocarbamate	Herbicide	4.1	19.7	0.05	83;154; 215
Fluridone	Pyridinone	Herbicide	1.9	58.7	0.05	328
Fenarimol	Pyrimidine	Fungicide	3.7	46.7	0.05	139; 219; 251
Terbacil	Uracil	Herbicide	1.9	24.2	0.05	161
Chlorpropham	Carbamate	Herbicide	3.4	20.2	0.05	127;171;213
Trifluralin	Dinitroaniline	Herbicide	5.3	19.9	0.05	264;306
Atraton	Triazine	Insecticide	2.7	21.8	0.05	169;196;211
Prometon	Triazine	Herbicide	4.3	21.9	0.05	168;210;226
Simazine	Triazine	Herbicide	2.2	22.5	0.05	186;200;203
Atrazine	Triazine	Herbicide	2.6	22.5	0.05	172;200;230
Propazine	Triazine	Herbicide	2.9	22.7	0.05	231
Pronamide	Amide	Herbicide	3.4	23.2	0.05	173;175;254
Simetryn	Triazine	Herbicide	2.6	26.2	0.05	213
Metribuzine	Triazine	Herbicide	1.6	26.6	0.05	198
Alachlor	Chloroacetamide	Herbicide	2.9	25.9	0.05	160;188
Ametryn	Triazine	Herbicide	3	26.3	0.05	213;227
Terbutryn	Triazine	Herbicide	3.6	26.9	0.05	170;185;;242
Napropamide	Amide	Herbicide	3.3	32.6	0.05	128;171;271
Metolachlor	Chloroacetamide	Herbicide	2.9	28.0	0.05	162;238
Triadimefon	Triazole	Fungicide	3.2	28.4	0.05	208;210
Diphenamid	Amide	Herbicide	2.2	29.4	0.05	166

MGK-264	Dicarboximide	Insecticide	3.7	29.0	0.05	164
Butachlor	Chloroacetamide	Herbicide	4.5	31.6	0.05	176;188
Norflurazon	Pyridazinone	Herbicide	2.3	38.8	0.05	102;145;303
Hexazinone	Triazine	Herbicide	1.2	39.4	0.05	171
Alpha-BHC	Organochlorinated	Insecticide	3.8	13.6	0.05	181;183
Endrin	Organochlorinated	Insecticide	5.6	27.0	0.05	281

* ON and OCl are respectively organonitrogen and organochlorine pesticides

Table 1: Targeted pesticides with their classification group, their function, their retention time, their limit of quantification (LOQ) and their qualifier ions.

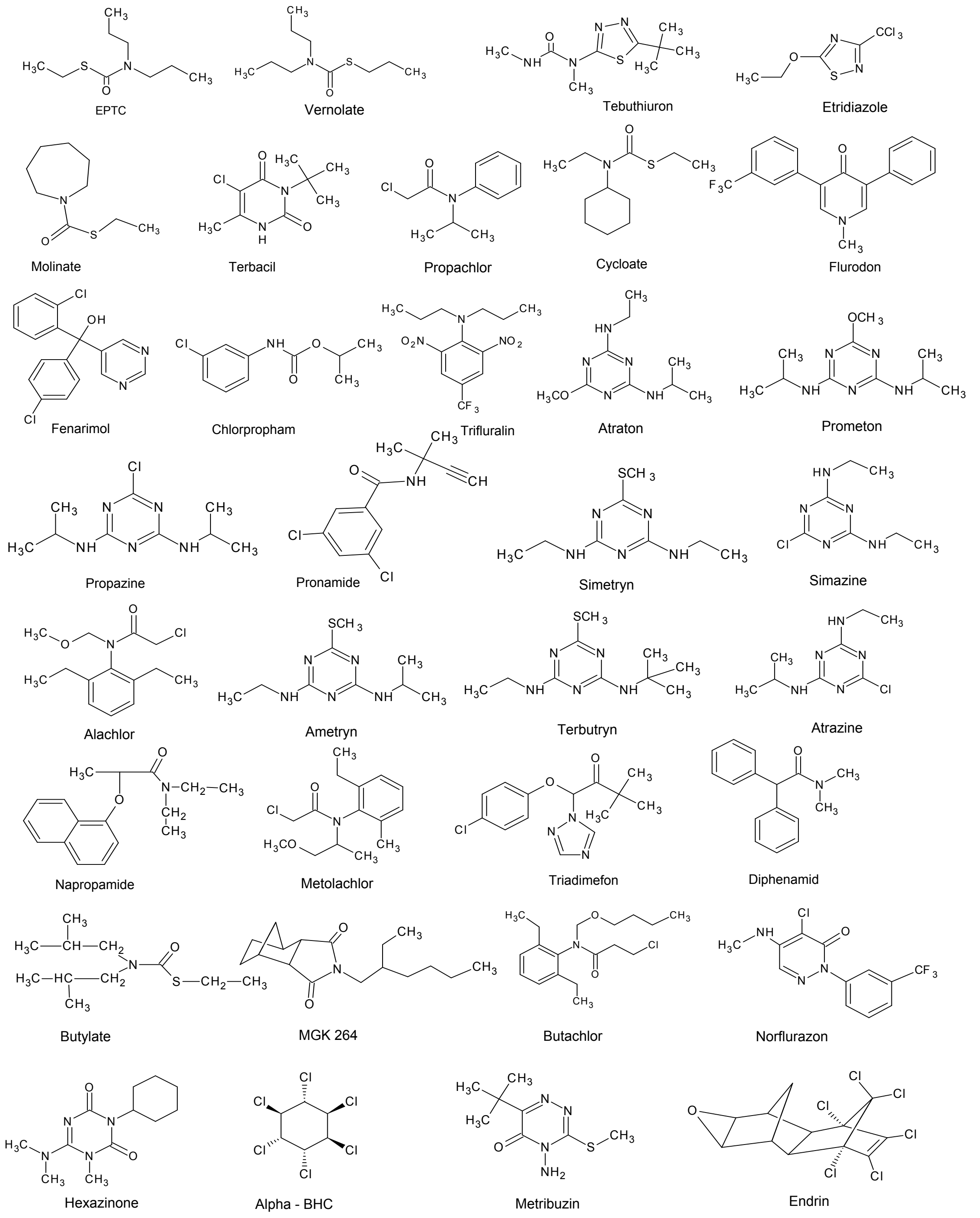


Figure 1: Structure of the thirty four targeted pesticides

2.2 Experimental procedure

750 mL of ultra-pure water was spiked with standard solution (at 3 µg.L^{-1}) of targeted compounds. pH was adjusted by addition of 1M aqueous hydrochloric acid or 0.5M methanolic solution of potassium hydroxide, and controlled using a Metrohm 713 pH meter (Herisau, Switzerland). Ionic strength was set up by addition of sodium chloride. Extraction was carried out using an SPE vacuum manifold system. The extraction consist of seven steps as following: (1) Conditioning step, the cartridge was conditioned with 5 mL of appropriated eluent according to the experiment set (Table 2), followed by 5 mL of methanol, (2) Equilibration step, 10 mL of ultra-pure water was added to wet the sorbent surface. (3) Percolation step, the sample was percolated into the cartridge. (4) After the sample was loaded, the cartridge was washed with 5 mL of ultra-pure water followed by 5 mL H_2O/MeOH (95/5 *v/v*). (5) Drying the cartridge with high purity nitrogen flow for 5 min. (6) Eluting with 2 x 5 mL (MeOH/eluent. 1/1 *v/v*). (7) Evaporating using the rotary evaporator until reaching a volume of 2 mL, then continuing evaporation under gentle nitrogen stream. (8) Fixing the final volume to 500 µL before GC-MS analysis.

2.3. Choice of operating variables and their variation levels

Numerous factors are known to influence significantly the efficiency of SPE extraction of pesticides from water. To increase the recovery of the studied compounds, pH must also be optimized according to targeted analytes chemical properties. Besides, some studies have reported that ionic strength can also influence the recovery of pesticides on the fact that the water solubility of polar organic compounds is diminished in high ionic strength aqueous media[46]. Likewise, nature of eluent and flow rate while elution can as well influence the extraction of pesticides. For example, Baugros et al. (2008)[45] and Guardia-Rubio et al. (2007)[47] reported that low flow rate increases interaction between the sorbent and targeted compounds thus leading to a better recovery.

In this study, hydrophilic-lipophilic balanced (HLB) polymer was selected as sorbent with the aim to cover the entire range of lipophilicity of targeted compounds (Log K_{OW} ranging from 1.2 to 5.6) [37-43]. Five variables that are known to affect the extraction efficiency were studied: sample flow rate, sample pH, elution flow rate, ionic strength (NaCl addition) and the nature of eluent. The selected variables in this study and their variation levels are summarized in table 2.

Variable	**Factor**	**Level**		
		- 1	**0**	**+1**
X1	Sample flow rate (mL.min^{-1})	2 - 4	5 - 7	8 - 10
X2	pH	3	6	9
X3	Elution flow rate (mL.min^{-1})	0.2	0.5	1
X4	Ionic strength (g.L^{-1} of NaCl)	0	50	100
X5	Eluent (1/1 *v/v*)	DCM/MeOH	AcOEt//MeOH	ACN/MeOH

Table 2: Summary of factors and their levels as coded and natural variables.

Three levels of variable have been studied, with sample flow rate ranging from 2 to 10 mL.min^{-1}, pH from 3 to 9, elution flow rate from 0.2 to 2 mL.min^{-1} and ionic strength from 0 to 100 g.L^{-1} of NaCl. Eluent is a qualitative variable; the solvent composition was performed with DCM/MeOH, AcOEt/MeOH and ACN/MeOH in a 1/1, *v/v* proportion.

2.4. Optimization strategy

Several models of experimental design such as factorial designs, central composite, Doehlert and simplex method can be applied[48-52]. Factorial designs are appropriate to evaluate principal effects as well as interactions between chosen factors. However, any experimental design remains empirical and requires replicated experiments to account for the variation and the uncertainty of measurements. Optimal designs reduce the costs of experimentation by allowing statistical models to be estimated with fewer experimental runs. In this work, 34 experiments were selected according to a D-optimality criterion[49] with the aim to estimate parameters without bias and with a minimum variance.

2.5. Mathematical model postulated: D-optimal design and exchange algorithm

The selection of the mathematical model constitutes the second step of the experimental design methodology. In this study, a second order polynomial model was postulated:

$$y = \beta 0 + \sum_i \beta i Xi + \sum_i \beta ii Xi^2 + \sum_{i,j} \beta ij Xi Xj + \varepsilon \quad (1)$$

X represents the model matrix or effect matrix with a N × p dimension (where N is the number of experiments and p is the number of coefficients of the model), y is the vector of the experimental responses, βi is the vector of the coefficients of model and ε is the vector of the experimental errors. When the model is adjusted to the experimental data, any experimental error is transmitted to the coefficients and to the significance of the factors. The estimation of the coefficients βi, βii and βij allows determination of the effects of both the factors and the interactions between them:

$$\beta = (X^t.X)^{-1} . X^t.y \quad (2)$$

(X^tX) is the information matrix and $(X^tX)^{-1}$ is the dispersion matrix. The D-optimality criterion tends to minimize the dispersion matrix corresponding to the variance, and conversely allows to maximize the determinant of the information matrix. For this study, the D-optimal design has been built through the exchange algorithm Fedorov[53,54] that is shown by natural and coded variables in Table 3.

Experiment	Coded variables					Natural variables				
	X1	X2	X3	X4	X5	Sample flow rate (mL.min^{-1})	pH	Elution speed (mL.min^{-1})	NaCl (g.L^{-1})	Eluent
1	+1	-1	-1	-1	DCM/MeOH	8 - 10	3	0.2	0	DCM/MeOH
2	-1	+1	-1	-1	DCM/MeOH	2 - 4	9	0.2	0	DCM/MeOH
3	+1	+1	+1	-1	DCM/MeOH	8 - 10	9	2	0	DCM/MeOH
4	+1	+1	-1	+1	DCM/MeOH	8 - 10	9	0.2	100	DCM/MeOH
5	+1	-1	+1	+1	DCM/MeOH	8 - 10	3	2	100	DCM/MeOH
6	-1	+1	+1	+1	DCM/MeOH	2 - 4	9	2	100	DCM/MeOH
7	-1	-1	-1	0	DCM/MeOH	2 - 4	3	0.2	50	DCM/MeOH
8	-1	-1	0	+1	DCM/MeOH	2 - 4	3	1.1	100	DCM/MeOH
9	-1	0	-1	+1	DCM/MeOH	2 - 4	6	0.2	100	DCM/MeOH
10	-1	0	+1	-1	DCM/MeOH	2 - 4	6	2	0	DCM/MeOH
11	0	-1	+1	-1	DCM/MeOH	5 - 7	3	2	0	DCM/MeOH
12	-1	-1	-1	-1	AcOEt/MeOH	2 - 4	3	0.2	0	AcOEt/MeOH
13	+1	+1	-1	-1	AcOEt/MeOH	8 - 10	9	0.2	0	AcOEt/MeOH
14	+1	-1	+1	-1	AcOEt/MeOH	8 - 10	3	2	0	AcOEt/MeOH
15	-1	+1	+1	-1	AcOEt/MeOH	2 - 4	9	2	0	AcOEt/MeOH
16	+1	-1	-1	+1	AcOEt/MeOH	8 - 10	3	0.2	100	AcOEt/MeOH
17	-1	+1	-1	+1	AcOEt/MeOH	2 - 4	9	0.2	100	AcOEt/MeOH
18	-1	-1	+1	+1	AcOEt/MeOH	2 - 4	3	2	100	AcOEt/MeOH
19	+1	+1	+1	+1	AcOEt/MeOH	8 - 10	9	2	100	AcOEt/MeOH
20	0	0	0	0	AcOEt/MeOH	5 - 7	6	1.1	50	AcOEt/MeOH
21	-1	-1	-1	-1	ACN/MeOH	2 - 4	3	0.2	0	ACN/MeOH
22	-1	+1	-1	+1	ACN/MeOH	2 - 4	9	0.2	100	ACN/MeOH
23	+1	-1	+1	+1	ACN/MeOH	8 - 10	3	2	100	ACN/MeOH
24	-1	-1	+1	0	ACN/MeOH	2 - 4	3	2	50	ACN/MeOH
25	-1	+1	0	-1	ACN/MeOH	2 - 4	9	1.1	0	ACN/MeOH
26	-1	0	+1	+1	ACN/MeOH	2 - 4	6	2	100	ACN/MeOH
27	+1	-1	0	-1	ACN/MeOH	8 - 10	3	1.1	0	ACN/MeOH
28	+1	+1	-1	0	ACN/MeOH	8 - 10	9	0.2	50	ACN/MeOH
29	+1	+1	0	+1	ACN/MeOH	8 - 10	9	1.1	100	ACN/MeOH
30	+1	0	-1	-1	ACN/MeOH	8 - 10	6	0.2	0	ACN/MeOH
31	0	-1	-1	+1	ACN/MeOH	5 - 7	3	0.2	100	ACN/MeOH
32	0	+1	+1	-1	ACN/MeOH	5 - 7	9	2	0	ACN/MeOH
33	0	0	0	0	ACN/MeOH	5 - 7	6	1.1	50	ACN/MeOH
34	0	0	0	0	ACN/MeOH	5 - 7	6	1.1	50	ACN/MeOH

Table 3: Mathematical design acquired from the model with the coded and real values of each variable.

2.6. GC-MS analysis

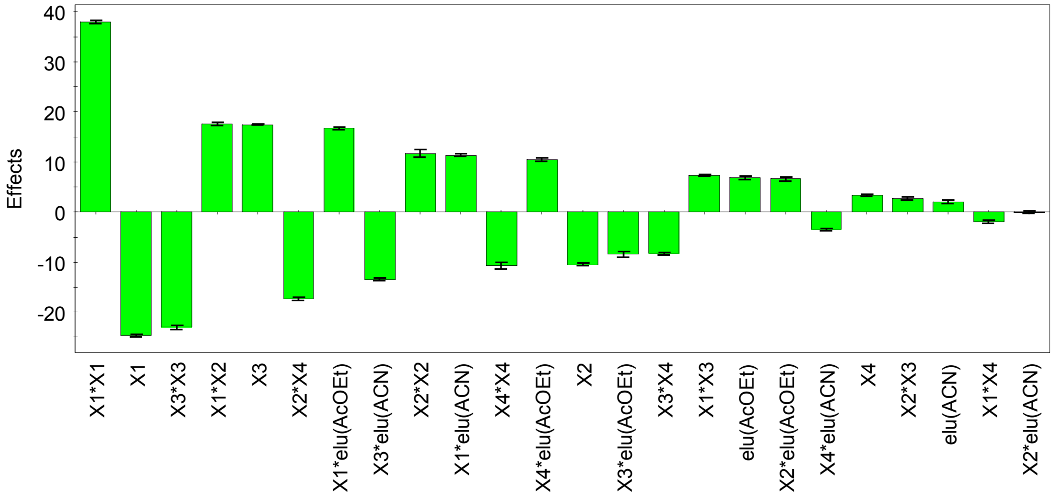
Analyses of pesticides were carried out using a Varian 3900 gas chromatograph, equipped with a deactivated fused-silica guard column (5 m x 0.25 µm i.d.) and a low polarity si-arylene ZB-XLB capillary column (60 m x 0.25 mm i.d. x 0.25 µm film thickness), coupled with an Ion Trap Saturn 2000 Mass Spectrometer (Varian Inc.) operating either in selected ion storage (SIS) or in full scan (FS) mode. Helium was used as carrier gas with a constant flow rate of 1 mL.min^{-1}. Samples were injected in the splitless mode at 280°C and the injector was purged with helium after 1 min. The temperature of the GC was programmed as follows : initial temperature 80°C, held for 1 min, 10°C.min^{-1} ramp to 170°C then 4°C.min^{-1} ramp to 230°C and finally 3°C.min^{-1} to 280°C and held for 2 min. The total analysis time was 43.67 min. The transfer line and the ion trap mass spectrometer were respectively held at 280°C and 220°C. Identification of each compound was done on the basis of the retention time and the mass spectrum obtained from chromatogram of standard solution acquired in full scan mode. Quantification was then performed in the SIS mode using the most abundant ions (Table 1).

3. Results and discussion

The D-optimal design was used for the optimization of the five selected parameter susceptible to impact solid-phase extraction efficiency for various pesticides in water.

3.1. Analysis of designed experiments: Effect of the factors

Results were explored using statistical and graphical analysis software (Modde 5.0 Umetrics. Sweden). This software was used for regression analysis of the data obtained from the set of 34 experiments and to estimate the coefficients of regression equation. Analysis of variance (ANOVA), squared and interaction terms were applied to test the significance of each term in the equation. The coefficient of correlation R^2 represents the fraction of the response variation explained by the model whereas Q^2 accounts for the fraction of the response variation that can be predicted by the model. The effects of the different factors on the extraction yield of 34 pesticides are displayed on figure 2.



* *X1, X2, X3 and X4 are respectively sample flow rate, pH, elution speed and NaCl.*

Figure 2: Effects of different factors on the extraction of 34 pesticides. Statistical values are: regression coefficient, R^2 = 1.00; adjusted coefficient of regression, R^2 Adj = 1.00; reliability of the mathematical model, Q^2 = 0.999 and mean square residuals, RSD = 0.0165.

These results indicate that sample flow rate (X1) has a negative influence on the extraction yield of the targeted compounds. This observation is in accordance with literature reviews where extraction efficiency increases when working at low flow rate[10,55,56]. Consequently, this variable should be fixed at its lower level (2 - 4 $mL.min^{-1}$). Concerning the pH (X2), it can also be seen a negative effect. Likewise, the interaction of pH with ionic force (X4) is negative while interactions with X5(ACN), X5(AcOEt) and the elution flow rate (X3) are not significant. Given the neutral character of the studied analytes, neutral pH values should be prefered[10,56]. Elution speed (X3) shows a positive influence that can significantly affect the extraction efficiency. Meanwhile, interactions between X3 with sample flow rate (X1), pH (X2), ionic force (X4) and X5(ACN), X5(AcOEt) are not significant. This parameter will be also discussed later by analyzing the surface response curves in the next part. The main effect of ionic strength (X4) is weakly positive. However, the interactions between X4 and the other factors do not allow evident conclusion, and this part will be also discussed later by analyzing the surface response curves. Concerning the eluent nature, among the three mixtures of eluent employed, AcOEt/MeOH generally lead to better extraction yields.

3.2. Analysis of designed experiments: Surface response curve

Analysis of factors indicates that low sample flow rate gives better recovery. In the same manner, working at neutral pH and using AcOEt/MeOH for elution have proved better extraction yields. Setting up the sample flow rate at its low level (2-4 mL.min^{-1}) combined with a neutral pH level (pH = 6) and AcOEt/MeOH as eluent, the response surface curve (figure 3) shows the influences of the elution speed and the ionic strength (NaCl) on the extraction efficiency.

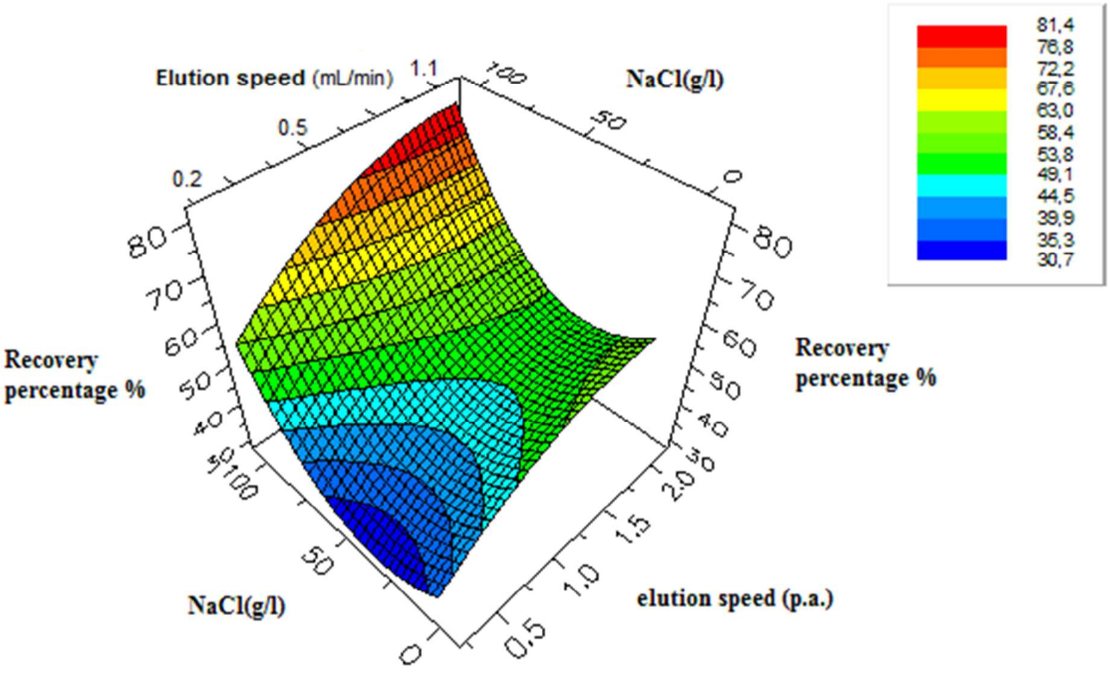


Figure 3: The surface response curve of targeted pesticides. The fixed parameters are: pH 6, sample flow rate: 2-4 mL.min^{-1}; and a mixture of AcOEt/MeOH (1/1 *v/v*) as eluent.

It can be seen that an increase of the ionic strength influences positively the extraction efficiency. NaCl (X4) should also be set up at its maximum level (100 g.L^{-1}) for an optimal extraction. This observation is in accordance with other studies focused on the impact of ionic strength for pesticide extraction efficiency. Indeed, Bagheri et al. (2000)[10] reported that the highest recovery for the extraction of diazinon was obtained when adding 5 % of NaCl, while Tolosa et al. (1996)[57] used 60 g.L^{-1} of NaCl for multi-residues extraction of organophosphorus and organochlorine pesticides. According to Font et al. (1993)[9], it can be admitted that an increase in the ionic strength of aqueous samples leads to weakening the interactions between undissociated molecules and water, thus resulting in an increasing of the extraction efficiency. Concerning the elution speed, good recoveries were obtained with the value ranging from 0.4 to 1 mL.min^{-1}. Since desorption of targeted analytes should be greater with a moderate flow rate, it was decided to set up this factor at its middle level (0.5 mL.min^{-1}).

The defined model predicts the optimal solid-phase extraction conditions as: sample flow rate at low level (2-4 mL.min^{-1}), pH = 6, AcOEt/MeOH (1/1 *v/v*) as eluent, NaCl = 100 g.L^{-1} and elution speed at 0.5 mL.min^{-1}. By setting up these conditions, the concentration of targeted pesticides (34 compounds) can be calculated by mathematical model as below:

$$\mathbf{y} = 65.56 - 3.97X1 - 1.93X2 + 4.53X3 + 6.91X4 + 18.94X1^2 + 5.85X2^2 - 11.49X3^2 - 5.32X4^2 + 8.78X1X2 + 3.66X1X3 - 0.97X1X4 + 1.37X2X3 - 8.68X2X4 - 4.11X3X4$$

3.3. Optimal conditions and method validation

The optimized method was firstly validated by comparing the average of experimental values with predicted values obtained from the mathematical models. The procedure was validated for 15 pesticides using both ultra-pure water and river water (Figure 4). The mean of the predicted extraction yield for 32 pesticides initially chosen was 91.0 %. Extraction yields of the 15 selected pesticides obtained from spiked ultra-pure water were ranging from 43 % for butachlor to 132 % for molinate with a mean value of 97 % (Table 4). Low recovery of butachlor compared to other chloroacetamides compounds such as alachlor (recovery of 105 %, log K_{OW} = 2.9), propachlor (recovery of 103 %, log K_{OW} = 2.4) or metolachlor (recovery of 99 %, log K_{OW} = 2.9) can be attributed to the higher hydrophobicity of butachlor (log K_{OW} = 4.5) due to its butyl chain. On the other hand, the modest recovery of carbamate chlorpropham (65 %) compared to the one obtained for the thiocarbamate molinate could be associated to the higher hydrogen bonding capacity of chlorpropham (both H-bond donor and acceptor). Our results were comparable to those found in literature. Kouzayha et al. (2012)[56] reported the extraction yield of 106 %, 106 % and 116 for respectively alpha-lindane, alachlor and propizamide while the developed method gave recoveries of 82 %, 105 % and 107 % respectively for the same compounds. Robustness of the method was also tested by applying the analytical procedure to spiked river water samples originated from the Canche River, in northern France. These additional analyses were performed in triplicate. Non-spiked river water revealed no trace of the targeted analytes. Extraction yields were found to vary from 54 % for butachlor to 104 % for endrin with a satisfactory mean recovery of 83 %.

Compound	Extraction yield in ultra-pure water (%)	Extraction yield in River water (%)
Alachlor	105	79
Ametrin	131	101
Atraton	78	94
Butachlor	43	54
Chlorpropham	65	65

Metolachlor	99	76
Molinate	132	74
Napropamide	100	73
Prometon	100	91
Propachlor	103	91
Pronamide	107	89
Terbutryn	82	89
Triadimefon	105	77
Alfa lindane	82	85
Endrin	119	104
Mean value	**97 ± 23**	**83 ± 13**

Table 4: Average recoveries of the 15 pesticides validated in this work.

5. Conclusion

This work presents an optimized SPE strategy using HLB cartridge followed by GC-MS analysis. The method was optimized for determination of fifteen pesticides embedded with various structural characteristics (triazine, carbamate, and thiocarbamate as well as organochlorine compounds). The validated method could be considered as a green method regarding the volume of eluents used, and by the fact that ethyl acetate can be regarded as an environmentally friendly solvent. The other benefit of the developed procedure results in its simplicity and is the possible application for routine analyses. Optimized conditions include percolation of 750 mL of filtered-water samples at natural pH on HLB cartridges at 2-5 $mL.min^{-1}$ flow rate. The ionic strength of the sample is controlled by the addition of sodium chloride (100 $g.L^{-1}$), whereas elution should be performed at a moderate speed fixed at 0.5 $mL.min^{-1}$ with AcOEt/MeOH (1/1 v/v) as eluent. This method was optimized using a mathematical model, D-optimal designs. The D-optimal matrix designed in this work clearly reveals the effects of different important parameters affecting the extraction efficiency in detail and their interactions. Many aspects of these results confirm the previously reported experimental data. Using the D-optimal method, not only the optimum extraction conditions for different types of pesticides were achieved, but also a great deal of information about the effects of each factor on the recovery could be obtained while the minimum number of experiments is performed.

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