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# Factorial design optimization of experimental variables in preconcentration of carbamates pesticides in water samples using solid phase extraction and liquid chromatography–electrospray-mass spectrometry determination

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## ABSTRACT

An experimental design was applied for the optimization of extraction process of carbamates pesticides from surface water samples. Solid phase extraction (SPE) of carbamates compounds and their determination by liquid chromatography coupled to electrospray mass spectrometry detector were considered. A two level full factorial design $2^k$ was used for selecting the variables which affected the extraction procedure. Eluent and sample volumes were statistically the most significant parameters. These significant variables were optimized using Doehlert matrix. The developed SPE method included 200 mg of C-18 sorbent, 143.5 mL of water sample and 5.5 mL of acetonitrile in the elution step. For validation of the technique, accuracy, precision, detection and quantification limits, linearity, sensibility and selectivity were evaluated. Extraction recovery percentages of all the carbamates were above 90% with relative standard deviations (R.S.D.) in the range of 3–11%. The extraction method was selective and the detection and quantification limits were between 0.1 and 0.5 $\mu g\ L^{-1}$, and 1 and 3 $\mu g\ L^{-1}$, respectively.

## 1. Introduction

The use of carbamates insecticides on potato, citrus, coffee and other crops is a common practice that can result in the contamination of both surface and ground waters. Carbamates are included in the list of the United States Environmental Protection Agency (EPA) [1] for prioritizing control of their application. Some compounds of this group such as carbaryl, carbofuran, aldicarb and among these aldicarb is highlighted as the principal target on the list of the controlled pesticides by the EPA due to its elevated toxicity [1].

Determination of these carbamates in different matrices such as water [1,2], soil [3–5], air [6], biological fluids [7] and foods [8,9] is well defined in the literature. This reflects the importance of their determination since intoxication by these compounds affects the nervous system by interruption of an enzyme which regulates acetylcholine, a neurotransmitter capable of causing the death of the organism [10].

Because the carbamates concentration levels are very low in water samples, sensitive analytical techniques are required to obtain low detection limits. Enrichment separation approaches including solvent extraction, solid phase extraction (SPE) and solid phase microextraction (SPME) have been commonly used to improve the instrumental limits of detection [11–18]. In this sense solid phase extraction procedures have a very important role in preconcentration studies due to their simplicity, high enrichment factors, environmental friendliness …. However, one of the challenging and time consuming steps with such preconcentration techniques has been the optimization of several factors that affect the extraction procedure. In most cases, a classical one-at-a-time approach is applied, which may result in wrong conclusions if there are some interactions among the different factors [19]. In the recent years multivariate techniques have been used for optimization of analytical methods. These techniques allow more than one

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variable to be optimized simultaneously and have several advantages, such as speed of analysis, practicality, economy and reduction of number of experiments that need to be carried out [20–25]. In addition, these methods are able to generate mathematical models that permit to estimate the relevance as well as statistical significance of the factors' effects on the processes and also evaluate the interactions' effects among the factors. Factorial design is one of the available statistical processes for multivariate optimization and is widely applied in chemistry due to its usefulness in the identification of the significant variables and the best conditions of an experiment. However, in order to determine the real functionality established among the analytical response and the significant factors, second order designs are used.

In this work, a preconcentration procedure using solid phase extraction for the determination of carbamates pesticides in water samples by liquid chromatography coupled to electrospray mass spectrometry (LC–ESI-MS) is proposed. A two level full factorial design was used to evaluate the experimental variables including eluent volume, sample volume and adsorbent mass. The experiments for the optimization were performed according to Doehlert matrix. Responses of three factors are presented in the entire experimental studied field. To the best of our knowledge, this is the first time that chemometric approach was applied for SPE of carbamates pesticides from water samples.

## 2. Experimental

### 2.1. Chemicals and materials

The HPLC grade-water was obtained by purification of demineralized water in a Milli-Q system (Millipore, Elix). Methanol, gradient grade, and acetonitrile, LC grade, were purchased from LAB SCAN (Ireland). The certified pure products were purchased from Supelco. The purity of all standards was a minimum of 99%. Standard stock solutions of 1000 μg mL$^{-1}$ were prepared by weighing and dissolving 20 mg of each compound in 20 mL of methanol. These solutions were stored at 4 °C. The analyzed pesticides carbamates were: aminocarb, baygon, carbofuran, carbaryl, methiocarb, baycarb, landrin and zectran. The chemical structures of all studied compounds are shown in Fig. 1.

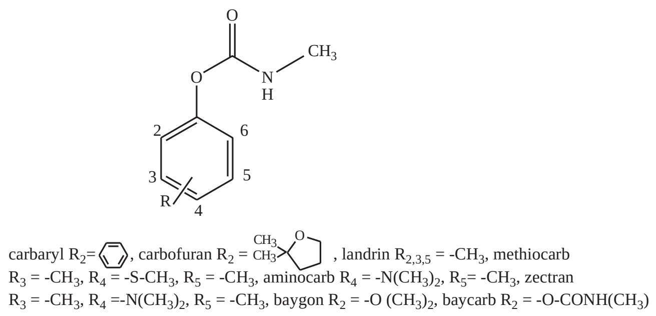
Standard mixtures were prepared by appropriate dilution of a mixed stock solution with methanol ranging from 0.005 to 1 μg mL$^{-1}$.

Silica-based sorbents with octadecyle functional group Bond Elut Jr. C18 were acquired from Varian.

### 2.2. Apparatus

The LC–MS analyses were performed using a Perkin Elmer LC system consisting of a 200 quaternary pump; a Rehodyne injection valve (model 7125) and PESciex API 2000 triple quadrupole mass spectrometer equipped with ESI ionization source.

The HPLC column was Xterra, 250 × 4.6 mm i.d., 5 μm particulate size. The LC–MS system was connected to analyst station for recording chromatograms.



carbaryl $R_2$=, carbofuran $R_2$ = , landrin $R_{2,3,5}$ = -$CH_3$, methiocarb $R_3$ = -$CH_3$, $R_4$ = -S-$CH_3$, $R_5$ = -$CH_3$, aminocarb $R_4$ = -N$(CH_3)_2$, $R_5$= -$CH_3$, zectran $R_3$ = -$CH_3$, $R_4$ =-N$(CH_3)_2$, $R_5$ = -$CH_3$, baygon $R_2$ = -O $(CH_3)_2$, baycarb $R_2$ = -O-CONH$(CH_3)$

**Fig. 1.** Structures of studied phenyl *N*-methylcarbamtes.

### 2.3. Chromatographic conditions

An acetonitrile–water binary gradient of 20–100% acetonitrile in 40 min was used. The flow rate of the mobile phase was 1 mL min$^{-1}$. The column effluent was split, allowing only 100 μL to enter the mass spectrometer.

### 2.4. Mass spectrometric analysis

The electrospray mass spectrometry (ES–MS) interface was operated in positive mode under the conditions of 325 °C gas temperature, 30 psi drying gas pressure, 35 psi nebulizer gas pressure, 70 psi additional gas pressure and 5000 V of capillary voltage. Full-scan LC–MS chromatograms were obtained by scanning from *m*/*z* 50 to 400 amu. Time scheduled SIM of the most abundant ion of each compound was used for quantification. All MS–MS experiments were performed using $N_2$ as the collision gas at a collision cell pressure of 2 mTorr and with collision energy ranging between 5 and 20 eV. Mass analysis was performed in MS–MS product ion mode with the first quadrupole locked on the *m*/*z* value corresponding to the molecule ion of the target compound, with the second quadrupole known as the collision cell either locked on a characteristic product ion *m*/*z* or scanning from *m*/*z* 50 to ca.50 amu above the molecular mass of the target compound (product ion scan) and the third quadrupole that filters the product ions and allows ions of certain mass-to-charge ratio to pass through the detector.

### 2.5. Sample preparation

Samples of water fortified of the studied carbamates at concentrations of 5 μg L$^{-1}$ were added. SPE cartridges packed with C18 bonded silica were first conditioned with 6 mL of methanol, then with 7 mL of deionized water. The pH of samples was set at 5.5. The solutes were eluted with methanol. The eluent was collected in a graduated tube and concentrated, under stream of nitrogen with Kuderna-Danish to 1 mL.

## 3. Results and discussion

### 3.1. Effect of the experimental parameters on the extraction recovery by means of the experimental design methodology

In order to evaluate the influence of operating parameters on the extraction recovery of carbamates, four main factors were chosen: elution volume ($U_1$), sample volume ($U_2$), mass of adsorbent ($U_3$) and sorbent type ($U_4$). A two level full factorial design $2^k$ was carried out to determine the influence of these four selected factors and their interaction [26,27]. In these types of designs, variables ($k$) are set at two levels (minimum) and (maximum) normalized as ($-1$) and ($+1$). The experimental response ($Y$) associated to a $2^k$ factorial design (for four variables) is represented by a linear polynomial model with interaction

$$\begin{aligned}Y = b_0 + b_1X_1 + b_2X_2 + b_3 \times_3 + b_4X_4 + b_{12}X_{12} + b_{13}X_{13} + b_{14}X_{14}\\ + b_{23}X_{23} + + b_{24}X_{24} + b_{34}X_{34} + b_{123}X_{123} + b_{134}X_{134} + b_{234}X_{234}\\ + b_{124}X_{124} + b_{1234}X_{1234}\end{aligned}$$

where is the $Y$ the experimental response the $X_i$ the coded variable ($-1$ or $+1$) the $b_i$ the estimation of the principal effect of the factor $i$ for the response $Y$ and $b_{ij}$ estimation of interaction effect between factor $i$ and $j$ for the response $Y$.

The coefficients of the equation model were calculated in the experimental field listed in Table 1. The choice of the limits of the investigated region is referred to a previous work [16]. The experimental design and results are represented in Table 2. The experimental response ($Y$) studied was the extraction efficiency, expressed in terms of percentage of recovery ($R$%).

**Table 1**
Investigated variables and their levels studied in the $2^4$ fatorial design.

| Coded variables ($X_i$) | Factors ($U_i$) | Unit | Experimental field | Coded variables ($X_i$) |
|---|---|---|---|---|
| | | | Minimum value (−1) | Maximum value (+1) |
| $X_1$ | $U_1$: eluent volume | mL | 1 | 7 |
| $X_2$ | $U_2$: sample volume | mL | 50 | 500 |
| $X_3$ | $U_3$: adsorbent mass | Mg | 100 | 300 |
| $X_4$ | $U_4$: adsorbent type | – | C8 | C18 |

**Table 2**
Factorial experimental design, experimental plan and results.

| Experiment number | Experimental design | | | | Experimental plan | | | | Results, $Y$ ($R$%) |
|---|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $U_1$ | $U_2$ | $U_3$ | $U_4$ | |
| 1 | −1 | −1 | −1 | −1 | 1 | 50 | 100 | C8 | 42.80 |
| 2 | 1 | −1 | −1 | −1 | 7 | 50 | 100 | C8 | 65.28 |
| 3 | −1 | 1 | −1 | −1 | 1 | 500 | 100 | C8 | 25.80 |
| 4 | 1 | 1 | −1 | −1 | 7 | 500 | 100 | C8 | 52.13 |
| 5 | −1 | −1 | 1 | −1 | 1 | 50 | 300 | C8 | 52.16 |
| 6 | 1 | −1 | 1 | −1 | 7 | 50 | 300 | C8 | 85.28 |
| 7 | −1 | 1 | 1 | −1 | 1 | 500 | 300 | C8 | 39.39 |
| 8 | 1 | 1 | 1 | −1 | 7 | 500 | 300 | C8 | 60.89 |
| 9 | −1 | −1 | −1 | 1 | 1 | 50 | 100 | C18 | 44.17 |
| 10 | 1 | −1 | −1 | 1 | 7 | 50 | 100 | C18 | 77.39 |
| 11 | −1 | 1 | −1 | 1 | 1 | 500 | 100 | C18 | 30.31 |
| 12 | 1 | 1 | −1 | 1 | 7 | 500 | 100 | C18 | 55.13 |
| 13 | −1 | −1 | 1 | 1 | 1 | 50 | 300 | C18 | 53.87 |
| 14 | 1 | −1 | 1 | 1 | 7 | 50 | 300 | C18 | 88.41 |
| 15 | −1 | 1 | 1 | 1 | 1 | 500 | 300 | C18 | 31.46 |
| 16 | 1 | 1 | 1 | 1 | 7 | 500 | 300 | C18 | 67.83 |

According to the results obtained, the coefficients of the polynomial model were calculated using the NEMROD Software [28]

$$\begin{aligned} Y = {} & 54.519 + 14.524X_1 - 9.151X_2 + 5.393X_3 \\ & + 1.553X_4 - 0.896X_{12} + 1.167X_{13} + 1.595X_{14} \\ & - 0.867X_{23} - 0.737X_{24} - 1.071X_{34} - 0.328X_{123} \\ & + 0.441X_{134} + 0.009X_{234} + 0.075X_{124} + 1.606X_{1234} \end{aligned}$$

The effects and interactions of the various investigated factors are represented in Fig. 2(a). This figure shows that the most influential variables are eluent volume, sample volume and adsorbent mass. However, the adsorbent type and the interactions variables have negligible effects. Eluent volume has a positive effect on the studied response. To increase extraction recoveries, it is worth working with a high eluent volume (7 mL). Sample volume is the second most significant factor on the extraction efficiency of carbamates from water samples. Its effect is negative. In fact, when a water sample is applied to the cartridge, analytes are loaded on the sorbent until we have not reached the breakthrough volume. Breakthrough occurs when the capacity of the sorbent has been reached. Over the breakthrough volume analytes are eluted by the sample matrix. The mass of adsorbent has a positive effect ($b_3 = 5.39$).

Pareto analysis [29] gives more significant information to interpret these results. In fact, this analysis calculates the percentage effect of each factor on the response, according to the following relation

$$P_i = \left( \frac{b_i^2}{\sum b_i^2} \right) \times 100 \; (i \neq 0)$$

Fig. 2(b) represents the Pareto graphic analysis. Results show that eluent and sample volume volumes are the most determining factors on the extraction recovery. Therefore, 87.63% of the response is bringing by these two factors. The adsorbent mass represents 8.66% of the response.

### 3.2. Determination of optimal conditions for the solid phase extraction of carbamates pesticides

Doehlert matrix [30] was used to represent the responses of the three factors in the all experimental studied field. In fact, the Doehlert matrix presents a number of advantages such as an

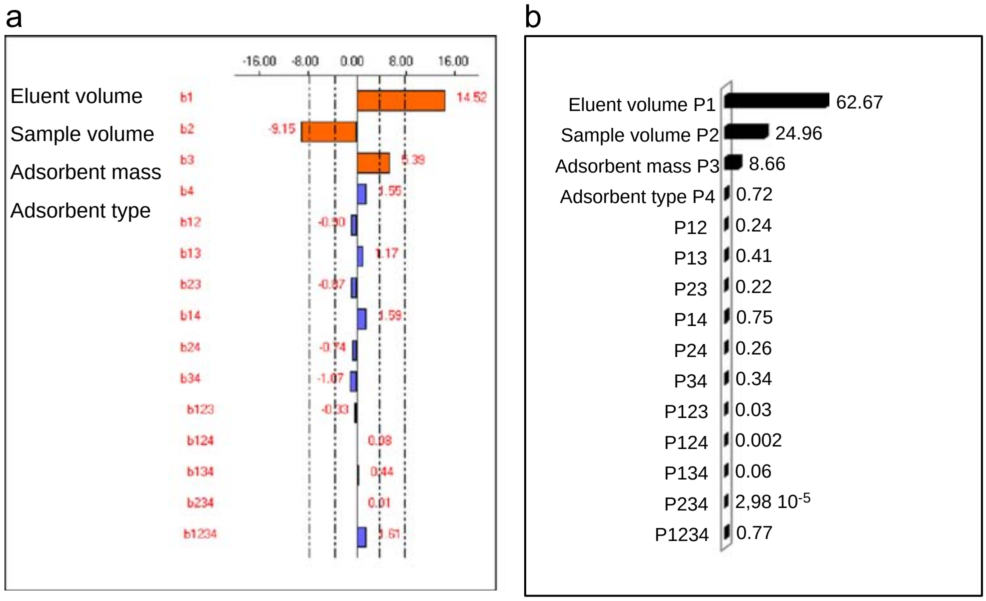


**Fig. 2.** (a) Graphic analysis of effects. (b) Graphical Pareto analysis.

uniform distribution of experimental points in the studied field, the ability to explore the whole of the experimental region, the usefulness of interpolating the response and the possibility of adding new factors defined on the basis of preliminary results factors without altering the quality of the matrix.

Three variables were regarded as factors that might potentially affect the extraction efficiency. The maximum and minimum values of each factor are listed in Table 3. In order to compare the effects of the different factors in the experimental field, concerned coded variables were used. The factors are given in the form of coded variables ($X_i$) without units in order to permit comparison of factors of different natures. The transformation of natural variables ($U_i$) into coded corresponding variables ($X_i$) is made on the basis of the following equation

$$X_i = \left[\frac{U_i - \overline{U}_i}{\Delta U_i}\right]\alpha$$

where is the $X_i$ the value taken by the coded variable $i$; $U_i$ the value taken by the factor $i$; $\overline{U}_i$ the value taken by the factor $i$ in the centre of the experimental field; $\Delta U_i$ the range of variation of the factor $i$, and $\alpha$ the maximum coded value of $X_i$: $X_1=1$; $X_2=0.866$; $X_3=0.816$.

$$\overline{U}_i = \frac{\text{upper limit of } U_i + \text{lower limit of } U_i}{2}$$

$$\Delta U_i = \frac{\text{upper limit of } U_i - \text{lower limit of } U_i}{2}$$

The response ($Y$) can be described by a second order model for predicting the response in all experimental regions from the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1{}^2 + b_{22}X_2{}^2 + b_{33}X_3{}^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where is the $b_i$ represents the estimation of the main effects of the factor $i$; $b_{ii}$ the estimation of the second order effects; and $b_{ij}$ the estimation of the interactions between factor $i$ and factor $j$.

The number of experiments required ($N$) is given by $N=k^2+k+1$ where $k$ is the number of variables. In the present case, $k=3$ and therefore the matrix was constituted of 13 experiments (Table 4). The levels of the independent variable (effective variables $U_i$) were calculated according to these following relations:

$$U_1 = 3X_1 + 4$$

$$U_2 = \frac{225}{0.866}X_2 + 275$$

$$U_3 = \frac{100}{0.816}X_3 + 200$$

Replicates at the central level of the variables are performed in order to validate the model by means of an estimate of experimental variance. The experiment at the centre (experiment number 13) was carried out three times (Table 5) in order to obtain an estimation of the experimental error.

**Table 3**
Experimental region investigated for SPE of carbamates.

| Coded variables ($X_i$) | Factors ($U_i$) | Unit | Experimental field | |
|---|---|---|---|---|
| | | | Minimum value (−1) | Maximum value (+1) |
| $X_1$ | $U_1$: eluent volume | mL | 1 | 7 |
| $X_2$ | $U_2$: sample volume | mL | 50 | 500 |
| $X_3$ | $U_3$: adsorbent mass | Mg | 100 | 300 |

**Table 4**
Doehlert matrix experiments and experimental results.

| Experiment number | Experimental design | | | Experimental plan | | | Results, $Y$ ($R$ %) |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $U_1$ | $U_2$ | $U_3$ | |
| 1 | 1.0000 | 0.0000 | 0.0000 | 7 | 275 | 200 | 78.12 |
| 2 | −1.0000 | 0.0000 | 0.0000 | 1 | 275 | 200 | 42.67 |
| 3 | 0.5000 | 0.8660 | 0.0000 | 6 | 500 | 200 | 63.89 |
| 4 | −0.5000 | −0.8660 | 0.0000 | 3 | 50 | 200 | 55.71 |
| 5 | 0.5000 | −0.8660 | 0.0000 | 6 | 50 | 200 | 86.83 |
| 6 | −0.5000 | 0.8660 | 0.0000 | 3 | 500 | 200 | 39.46 |
| 7 | 0.5000 | 0.2887 | 0.8165 | 6 | 350 | 300 | 51.94 |
| 8 | −0.5000 | −0.2887 | −0.8165 | 3 | 200 | 100 | 36.05 |
| 9 | 0.5000 | −0.2887 | −0.8165 | 6 | 200 | 100 | 65.36 |
| 10 | 0.0000 | 0.5774 | −0.8165 | 4 | 425 | 100 | 37.28 |
| 11 | −0.5000 | 0.2887 | 0.8165 | 3 | 350 | 300 | 43.07 |
| 12 | 0.0000 | −0.5774 | 0.8165 | 4 | 125 | 300 | 69.32 |
| 13 | 0.0000 | 0.0000 | 0.0000 | 4 | 275 | 200 | 67.26 |

**Table 5**
Repeated experiments in the centre of the investigated region.

| Eluent volume (mL) | Sample volume (mL) | Adsorbent mass (mg) | $R$ (%) |
|---|---|---|---|
| 4 | 275 | 200 | 67.26 |
| 4 | 275 | 200 | 67.84 |
| 4 | 275 | 200 | 67.56 |

According to these obtained results, the coefficients of the polynomial model were calculated using the NEMROD software [28]

$$Y = 67.553 + 20.579X_1 - 13.572X_2 + 5.234X_3 - 7.158X_1^2 - 5.722X_2^2 + -22.354X_3^2 - 3.863X_1X_2 - 11.151X_1X_3 - 5.425X_2X_3$$

Fig. 3 shows typical response surface profiles drawn versus the main factors eluent volume, sample volume and adsorbent mass and the three-dimensional representation of the same plots using the NEMROD [28] software. The analysis of the isoresponse curves at the chosen experimental field delimited by a circle show that the maximum of extraction recovery was obtained where the eluent volume and the sample volume are ranging respectively between 4 and 7 mL and 12 and 275 mL. Therefore, it is noted that extraction recovery is improved when the adsorbent mass is located in the centre of experimental region. Consequently the optimal working conditions are obtained when 143.5 mL of water sample are loaded on 200 mg of C-18 sorbent and then eluted with 5.5 mL of acetonitrile.

### 3.3. *Validation of the optimized method*

The optimized technique was validated in relation to the following properties: selectivity, linearity, detection and quantification limits, precision (repeatability and intermediary precision) and accuracy (recovery test and comparison of methods), according to recommendations of the International Conference on Harmonization (ICH) [31] and other publications [32].

The accuracy of the method was evaluated, with three repetitions, by recovery tests at three concentration levels and by comparison with an SPE extraction method cited in the literature [33]. The solid phase extraction method employs an octadecylsilane (C-18) as adsorbent and acetonitrile as eluent for analysis of carbamates in water.

#### 3.3.1. *Selectivity*

The selectivity of the method was verified by comparison of the chromatograms obtained from fortified water samples with those

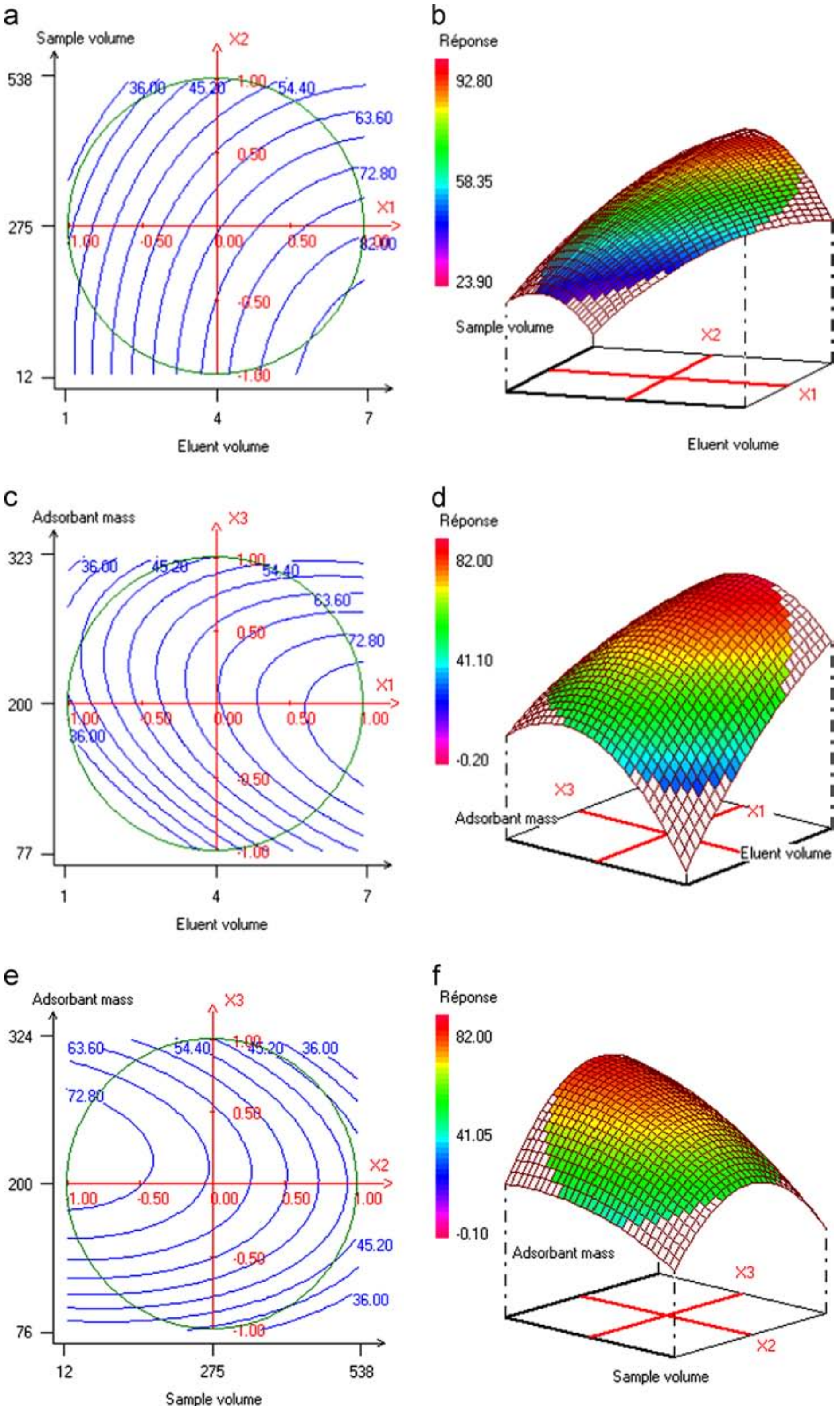


**Fig. 3.** (a) Contour plots of extraction recovery versus eluent volume (mL) and sample volume (mL) at a fixed mass adsorbent 200 mg; (b) corresponding three-dimensional plot; (c) contour plots of extraction recovery versus eluent volume (mL) and adsorbent mass (mg) at a fixed sample volume 275 mL; (d) corresponding three-dimensional plot; (e) contour plots of extraction recovery versus sample volume (mL) and adsorbent mass (mg) at a fixed eluent volume 4 mL; (f) corresponding three-dimensional plot; results obtained from Doehlert matrix (Table 4).

of samples free of carbamates (Fig. 4). The samples did not present peaks at the retention times of the carbamates, moreover, the chromatograms of the extracts presented satisfactory chromatographic resolution.

### *3.3.2. Detection and quantification limits*

The detection limit (LOD) of the proposed method was determined as being equal to at least 3 times the baseline signal (noise) obtained for water samples free of pesticides (blank), fortified with

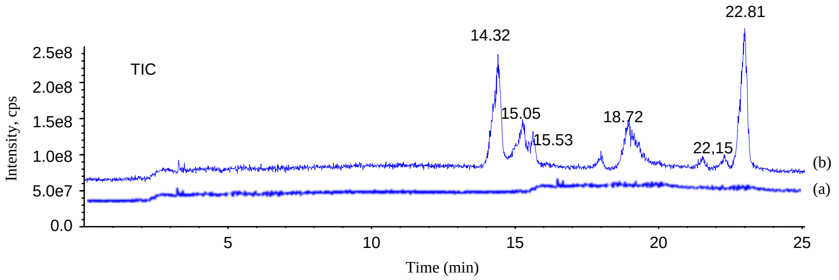


**Fig. 4.** Total ion current chromatograms obtained from the analysis of river water samples by SPE–LC–ESI-MS. (a) River water sample. (b) Spiked river water sample with carbamates at 10 μg L$^{-1}$.

**Table 6**
Correlation coefficients and equation of the straight line for the three analytical curves.

| | Pure solvent | | Blank of the matrix | | Superposition of the matrix | | LOD (μg L$^{-1}$) | LOQ (μg L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | $y=ax+b$ | $r^2$ | $y=ax+b$ | $r^2$ | $y=ax+b$ | $r^2$ | | |
| Aminocarb | $2.35\times10^9x+7.43\times10^7$ | 0.9984 | $2.12\times10^9x+5.43\times10^7$ | 0.9974 | $1.81\times10^9x+1.43\times10^7$ | 0.9990 | 0.5 | 3 |
| Baygon | $7.49\times10^8x+3.11\times10^7$ | 0.9979 | $6.94\times10^8x+1.14\times10^7$ | 0.9989 | $7.12\times10^8x+1.1\times10^6$ | 0.9997 | 0.2 | 1.5 |
| Carbouran | $3.39\times10^9x+2.43\times10^8$ | 0.9972 | $2.93\times10^9x+5.53\times10^8$ | 0.9981 | $3.19\times10^9x+4.23\times10^7$ | 0.9952 | 0.2 | 1.5 |
| Carbaryl | $1.95\times10^9x+2.43\times10^8$ | 0.9989 | $1.65\times10^9x+7.43\times10^8$ | 0.9990 | $2.01\times10^9x+22.43\times10^8$ | 0.9969 | 0.1 | 1 |
| Landrin | $5.35\times10^9x+9.43\times10^7$ | 0.9985 | $4.53\times10^9x+19.43\times10^7$ | 0.9975 | $5.05\times10^9x+59.43\times10^7$ | 0.9989 | 0.2 | 1.5 |
| Baycarb | $6.85\times10^9x+6.21\times10^7$ | 0.9991 | $6.58\times10^9x+12.41\times10^7$ | 0.9991 | $7.15\times10^9x+36.71\times10^7$ | 0.9990 | 0.5 | 3 |
| Methiocarb | $4.13\times10^9x+8.00\times10^8$ | 0.9975 | $3.73\times10^9x+28.60\times10^8$ | 0.9965 | $4.03\times10^9x+48.79\times10^8$ | 0.9987 | 0.2 | 1.5 |
| Zectran | $4.26\times10^9x+7.81\times10^8$ | 0.9988 | $4.62\times10^9x+7.54\times10^7$ | 0.9981 | $4.06\times10^9x+67.81\times10^8$ | 0.9992 | 0.2 | 1.5 |

carbamates between 1 and 1000 μg L$^{-1}$, submitted to the SPE technique and analyzed by LC–MS. The quantification limit (LOQ) was determined as being the signal at least 10 times greater than the noise signal. Detection limits were between 0.1 and 0.5 μg L$^{-1}$ and quantification limits were between 1 and 3 μg L$^{-1}$ (Table 6).

### 3.3.3. *Sensitivity and linearity*

The linearity of the detector response was evaluated by injecting solutions of each carbamate over a wide concentration range (0.005–1 mg L$^{-1}$). For each carbamate calibration curves were constructed. The detector proved to be linear for the eight studied compounds.

The operational range used was 0.005 to 1 mg L$^{-1}$. This curve was built using a blank matrix as well as pure solvent. The correlation coefficients were above 0.99 for the eight carbamates.

To check the matrix effect, the equations that describe three analytical curves (an analytical curve prepared by the dissolution of the carbamates in methanol, an analytical curve prepared by the dissolution of the carbamates in water extracts free of pesticides, and an analytical curve prepared from the superposed matrix) are presented in Table 6.

These analytical curves and the related linear regression data indicate that the response of the MS detector was linear for the eight compounds analyzed, with correlation coefficients greater than 0.99 for all three analytical curves. When analyzing the results, it can be observed that the three equations of the analytical curves are identical, considering their parallelism and superposition. Some recently published papers have demonstrated effects of the matrix on the determination of pesticide residues by gas chromatography [34] and high performance liquid chromatography with mass spectrometry [35,36]. These effects are principally observed in more complex matrices such as biological, food and drink matrices. The mechanism of the matrix effect on determinations by high performance liquid chromatography with ultraviolet detection is little known and there are no reports related to this subject in literature. Since water is a matrix free of proteins, carbohydrates and lipids, this effect is not significant, which can be proven by the similarity of the constructed analytical curves.

### 3.3.4. *Precision*

The precision of the method was determined in water samples fortified with carbamate standards (20 μg L$^{-1}$) and submitted to the SPE method, using five repetitions. The analytical curve utilized was that of the blank matrix. Intermediate precision was determined by evaluation the coefficients of variation from the SPE results of the water samples fortified with carbamates (20 μg L$^{-1}$) using determinations performed with five repetitions by the same analyst occurred on three different dates (1 day, 1week and 1 month). Precision of the SPE method for the studied carbamates in water samples, under repeatable conditions, was evaluated by estimation of the relative standard deviation or coefficient of variation (CV) for the five repetitions of the optimized analytical procedure. The extraction recovery percentages (%*R*) and coefficients of variation (CV) obtained are presented in Table 7. The results confirmed that the extraction technique and analysis of the carbamates in water did not suffer influences when performed in the same laboratory, by the same analyst, on different days. It can be stated that the extraction technique provides recovery percentages statistically equal (at 95% probability by the *t* test) and that the variation coefficients are below 11.3%.

### 3.3.5. *Accuracy*

The accuracy of the method was evaluated in triplicate by recovery tests at three concentration levels (20, 50 and 100 μg L$^{-1}$) and by comparison with other extraction methods. Therefore, the recovery tests were performed by extraction of the compound under study, present in water matrices, according to the proposed technique. The results obtained for extraction of the studied carbamates from water, as well as the respective coefficients of variation, are presented in Table 8. These results do not differ at a probability level of 95% by the *t*-test, showing that the

**Table 7**
Recovery percentages (%$R$) and coefficients of variation, CV (%), obtained from the application of SPE–LC–MS method with five repetitions, in water samples fortified with carbamates (20 $\mu$g L$^{-1}$) on different days.

| | Recovery (%); CV (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Aminocarb | Baygon | Carbouran | Carbaryl | Landrin | Baycarb | Methiocarb | Zectran |
| Day 1 | 91.0; 9.5 | 89.8; 3.2 | 95.3; 5.4 | 97.8; 4.6 | 96.3; 7.4 | 94.3; 8.5 | 98.9; 6.3 | 92.7; 9.5 |
| 1 Week | 88.9; 10.1 | 87.5; 4.6 | 94.7; 6.1 | 95.2; 4.9 | 96.7; 11.2 | 91.3; 8.1 | 99.5; 5.7 | 93.1; 8.3 |
| 1 Month | 89.8; 11.3 | 90.4; 4.8 | 95.3; 7.9 | 97.5; 4.3 | 98.1; 8.7 | 92.6; 7.4 | 97.8; 6.4 | 92.7; 7.9 |

**Table 8**
Recovery percentages and coefficient of variation (CV), obtained from the extraction of carbamates in fortified water samples, by the proposed and literature methods.

| | Recovery (%); CV[a] (%) | | | |
|---|---|---|---|---|
| | 20 $\mu$g L$^{-1}$ | 50 $\mu$g L$^{-1}$ | 100 $\mu$g L$^{-1}$ | (3 $\mu$g L$^{-1}$)[b] |
| Aminocarb | 91.0; 9.5 | 91.7; 6.4 | 92.6; 5.7 | 59.5; 12.7 |
| Baygon | 89.8; 3.2 | 90.9; 4.3 | 91.5; 8.3 | |
| Carbouran | 95.3; 5.4 | 97.2; 5.6 | 98.9; 3.6 | |
| Carbaryl | 97.8; 4.6 | 99.6; 6.9 | 98.5; 7.3 | |
| Landrin | 96.3; 7.4 | 97.5; 9.4 | 96.9; 8.4 | |
| Baycarb | 94.3; 8.5 | 95.8; 9.1 | 96.4; 8.2 | |
| Methiocarb | 98.9; 6.3 | 99.7; 9.5 | 99.8; 10.2 | |
| Zectran | 92.7; 9.5 | 94.5; 6.3 | 95.2; 4.3 | 96.8; 9.8 |

[a] Five replicates.
[b] Literature method with six replicates.

extraction recovery percentages are independent of the analyte concentration in the sample. Moreover, the coefficients of variation were lower than 10.2%. The results obtained by employing the SPE technique were compared with methods described in literature for extraction of carbamates [33]. These results are shown in Table 8.

The method described in the literature basically consists of a solid phase extraction of aminocarb, zectran and bendiocarb using C-18 cartridge (Agilent-Zorbax, 200 mg) as adsorbent and methyl-tertiobutylether as eluent. The results obtained from the recovery percentage for each carbamate by the literature method [33] and the proposed method were equivalent, showing no significant difference at the probability level of 95% by the *t*-test between these methods. However, aminocarb showed smaller extraction efficiency using the literature method, with recoveries of 59.5%.

### 3.4. Application to real samples

The potential of the method for the analysis of surface water samples has been demonstrated. In our laboratory, samples of tap and surfaces water from different sampling sites (river, dam and lagoon) all over Tunisia were collected for pesticides residue analysis. Recovery studies were performed on water samples spiked with 20 $\mu$g L$^{-1}$ of each carbamate. Average recoveries were greater than 89%. The relative standard deviations (R.S.D.) were in the range of 3–11%.

## 4. Conclusion

The experimental design methodology has been applied in this study to determine the optimal conditions of SPE procedure. The factorial designs demonstrated that eluent and sample volume are the most influent parameters on the SPE technique. Doehlert matrix was used to build a mathematical model. This model allowed the efficient optimization of the variables involved in the solid phase extraction procedure for the preconcentration of carbamates in water samples using ESI-MS detection. This methodology showed to be suitable in order to improve the sensitivity of ESI-MS detection. Analytical features such as limit of detection and accuracy were satisfactory and comparable to other methods reported in the literature.

## References

[1] U.S. E. P. A., National Survey of Pesticides in Drinking Water Wells, Phase II Report, EPA 570/9-91-020, 1992.
[2] D.A. Lambropoulou, T.A. Albanis, J. Chromatogr. A 1072 (2005) 55–61.
[3] L.L. El Atrache, S. Sabbah, J.P. Morizur, Talanta 65 (2005) 603–612.
[4] C. Sánchez-Brunete, A. Rodriguez, J.L. Tadeo, J. Chromatogr. A 1007 (2003) 85–91.
[5] C. Basheer, A.A. Alnedhary, B.S.M. Rao, R. Balasubramanian, H.K. Lee, J. Chromatogr. A 1216 (2009) 211–216.
[6] L. Sun, H.K. Lee, J. Chromatogr. A 1014 (2003) 165–177.
[7] H.-P. Li, J.-H. Li, G.-C. Li, J.-F. Jen, Talanta 63 (2004) 547–553.
[8] D.B. Barr, J.R. Barr, V.L. Maggio Jr., R.D. Whitehead, M.A. Sadowski, R.M Whyatt, L.L. Needham, J. Chromatogr. B 778 (2002) 99–111.
[9] K. Granby, J.H. Andersen, H.B. Christensen, Anal. Chim. Acta 520 (2004) 165–176.
[10] S. Bogialli, R. Curini, A. Di Corcia, A. Laganà, M. Nazzari, M. Tonci, J. Chromatogr. A 1054 (2004) 351–357.
[11] A. Sanusi, V. Guilet, M. Montury, J. Chromatogr. A 1046 (2004) 35–40.
[12] L.J. Krutz, S.A. Senseman, A.S.J. Sciumbato, J. Chromatogr. A 999 (2003) 103–121.
[13] B. Albero, C.S. Brunete, J.L. Tadeo, Talanta 66 (2005) 917–924.
[14] F.M. Lancas, Solid Phase Extraction, Editora Rima, São Carlos, 2004.
[15] Y.F. Picó, M.M.J. Ruiz, G.J. Font, Biochem. Biophys. Methods 70 (2007) 117–131.
[16] L.L. El Atrache, S. Sabbah, Can. J. Chem 81 (2003) 971.
[17] J.M. Soriano, B. Jiménez, G. Font, J.C Moltó, Crit. Rev. Anal. Chem. 31 (2001) 19.
[18] U.S. E.P.A. Method 531, EPA-600/4–85/054. United States Environmental Protection Agency, Washington, DC, 1985.
[19] P. Hashemi, S. Bagheri, M.R. Fathi, Talanta 68 (2005) 72–78.
[20] S.L.C. Ferreira, W.N.L. Dos Santos, C.M. Quintella, B.B. Neto, J.M. Bosque-Sandra, Talanta 63 (2004) 1061–1067.
[21] C.R.T. Tarley, W.N.L.D. Santos, C.M.D. Santos, M.A. Zezzi Arruda, S.L.C. Ferreira, Anal. Lett. 37 (2004) 1437–1455.
[22] M. Tuzen, M. Soylak, D. Citak, H.S. Ferreira, M.G.A. Korn, M.A. Bezerra, J. Hazard. Mater. 162 (2009) 1041–1045.
[23] M.A. Farajzadeh, M. Bahram, B.G. Mehr, J.A. Johnson, Talanta 75 (2008) 832–840.
[24] M.T.P.O. Castro, N. Baccan, Talanta 65 (2005) 1264–1269.
[25] S.L.C. Ferreira, M.A. Bezerra, W.N.L.D. Santos, B.B. Neto, Talanta 61 (2003) 295–303.
[26] J. Goupy, Les plans d'expériences, in: Techniques de l'ingénieur, Editions of T.I (ETI), vol. 4, pp. PE 230/1–PE 230/26.
[27] J. Goupy, La méthode des plans d'expériences, DUNOD, Paris (1996) 9–27.
[28] D. Mathieu, R.P.T. Luu, Software NEMROD, Université d'Aix-Marseille III, France, 1980.
[29] D.P. Haaland, Experimental Design in Biotechnology, Marcel Dekker Inc., New York, 1989.
[30] D.H. Doehlert, Appl. Statist 19 (1970) 231–239.
[31] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, 2001, Center for Drug Evaluation and Research, Rockville, MD, 〈http:/www.fda.gov/eder/guidance/4252fnl.htm〉.
[32] ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology, version 4, 2005, 〈http:/www.ich.org/cache/compo/276-254-1.html〉.
[33] F. Boujelbane, F. Oueslati, N. Ben hamida, Desalination 250 (2010) 473–478.
[34] G.P. Pinho, A.A. Neves, M.E.L.R. Queiroz, F.O. Silvério, Quim. Nova 32 (2009) 987–995.
[35] N. Mazzella, F. Delmas, B. Delest, J. Environ. Monit 11 (2009) 108–115.
[36] A. Kruve, A. Kunnapas, K. Herodes, I. Leito, J. Chromatogr. A 1187 (2008) 58–66.