Mixed-level orthogonal array design for the optimization of solid-phase extraction of some pesticides from surface water[☆]

H. Bagheri*, M. Saraji, M. Chitsazan, S.R. Mousavi, M. Naderi

Department of Chemistry, Sharif University of Technology, PO Box 11365-9516, Tehran, Iran

Abstract

An orthogonal array design (OAD), $OA_{32}(4^1 \times 2^{28})$, was employed as a chemometric method for the optimization of the solid-phase extraction (SPE) of atrazine, diazinon, ametryn and fenthion in surface water. Seven parameters: the type of eluting solvent, type of sorbent, flow-rate of eluting solvent, sample pH, sample volume, elution volume, addition of modifier and flow-rate of water sample were studied and optimized by a mixed-level OAD. The effects of these factors and some two-variable interactions on the recovery of the pesticides were quantitatively evaluated by the analysis of variance and percentage contribution techniques. The final optimized condition was employed for the SPE of selected micro-organic pollutants from Karoun river water, south of Iran. Atrazine and ametryn were tentatively identified and determined at the 0.7 and 0.9 μg l^{-1} level, respectively. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Orthogonal array design; Chemometrics; Solid-phase extraction; Optimization; Water analysis; Pesticides

1. Introduction

Environmental pollution is a worldwide problem in modern society. Water is a very important constituent of the ecosystem on the earth. The importance of water quality, preservation and improvement constantly is being increased. Pesticides, a major type of pollutants, are being used increasingly in agriculture and other areas.

Due to the complexity of the samples in which these pesticides should be determined, chromatographic methods are significantly important, particularly gas chromatography (GC) and high-performance liquid chromatography (HPLC) are pre-

ferred methods. In common procedures for water analysis, however, pesticides are isolated and preconcentrated from the aquatic matrix before chromatographic measurements. In general, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are adopted. The former is based on solvent partitioning in separating funnels, which is time consuming and requires large volumes of costly and toxic solvents. Besides, low sensitivity and selectivity are other disadvantages. Considerable attention is, therefore, being paid to SPE as a way to isolate and concentrate desired components from a sample matrix and has shown to be a suitable alternative for manual LLE. SPE offers advantages such as time saving, solvent reduction, elimination of emulsions and a high potential for automation [1–3].

GC and HPLC, subsequent to appropriate enrichment processes, have become the procedures of

choice for separating analytes of interest. GC is a very efficient technique with high resolving power and sensitivity and many specific detectors have been constructed to improve the selectivity. In conventional pesticides analysis, HPLC is generally limited to a few pesticides such as thermally labile and polar pesticides not amenable to GC.

Often SPE methods involve the investigation of many variables, which may affect the efficiency of extraction. Selection of these variables and their levels is critical. Several statistical techniques such as simplex optimization and factorial design were employed for the optimization of analytical methods. Factorial design has some advantages over simplex optimization in that global optimum can be provided, large amounts of quantitative information can be extracted and both discrete and continues factors can be estimated. One obvious disadvantages of the factorial design is the large number of experiments required when several variables are examined. However, the number of the experiments can be considerably reduced by the use of OAD [4,5].

Many researchers, already working on the development of SPE methods for the extraction of pesticides [6–8] from various samples, utilized factorial design to optimize important variables, which affect the recovery of pesticides. In their approaches five and/or four variables at two levels were investigated. Factors such as sample pH, eluting solvent, SPE sorbent, addition of an organic modifier to the sample and ionic strength were studied. Also, recently, Wan et al. [9] have employed OAD for the optimization of SPE of 30 pesticides from water samples. They used three $OA_7(2^7)$ matrices to study some variables on the recovery of extraction.

The theory and methodology of OAD, as a chemometric method for the optimization of the analytical procedure, have been described in detail elsewhere [4,5,10–15]. In this work, the effects of the type of eluting solvent, the type of sorbent, the flow-rate of eluting solvent, sample pH, sample volume, elution volume, addition of an modifier and flow-rate of water sample on the recovery of the pesticides were studied and optimized by a mixed-level OAD with an $OA_{32}(4^1 \times 2^{28})$ matrix. The final optimized condition was employed for the SPE of selected micro-organic pollutants from Karoun river water, south of Iran. Atrazine and ametryn were identified and determined at sub-μg l^{-1} level.

2. Experimental

2.1. Chemicals

The pesticides studied were obtained from Riedel-de Haen (Seelze, Germany). All of the solvents were HPLC-grade and pyren was used as an internal standard and purchased from Merck (Darmstadt, Germany).

Stock standard solutions of 1000 mg l^{-1} of each compound and the internal standard were prepared in methanol and stored at 4°C. Working standard solutions were prepared by diluting the stock solutions with ethyl acetate and were stored in the same manner.

2.2. Instrumentation

A Perkin-Elmer liquid chromatograph model 601 equipped with two reservoirs was modified for delivering the aqueous sample and preconcentration. SPE was carried out on a 10 mm×2 or 3 mm I.D., stainless steel precolumn packed with octadecyl bonded silica C$_{18}$ (Analytichem, Harbor city, CA, USA) or 15–25 μm PLRP-S (Polymer laboratories, Church Stretton, UK) styren divinylbenzene copolymer.

A gas chromatograph model Chrompack CP 9001 equipped with a flame ionization detector (FID) and a split/splitless injector with a split ratio of 1:100 was employed. Chromatographic separation was performed on a 10 m×0.25 mm I.D. CP-Sil 5CB, 100% dimethyl polysiloxane, capillary column from Chrompack with a 0.12-μm film thickness. The initial column temperature was set at 50°C for 2 min, then increased to 125°C (5°C/min, 1 min hold), programmed to 155°C (2°C/min, 1 min hold), increased to 210°C (5°C/min, 1 min hold) and then finally increased to 270°C at 10°C/min. The injector and detector temperatures were set at 260°C and 280°C, respectively. The flow-rate of the nitrogen carrier gas was 0.8 ml min^{-1}.

2.3. Procedure

The SPE precolumn was cleaned and conditioned by passing 5 ml of HPLC-grade methanol at 0.5 ml/min, then 10 ml of distilled water was used to

wash the cartridge at 1 ml min^{-1}. For calculating the recovery, a sample of water (pH 3 or 7), containing four pesticides at 2–10 μg l^{-1} was pumped at 3 or 6 ml min^{-1}. Subsequent to the enrichment procedure, 10 ml of pure water was used to flush the precolumn. Then, any residual water was removed by purging the nitrogen stream for 20 min. Retained compounds on the cartridge was desorbed with 1 or 3 ml eluting solvents at 0.2 or 1 ml min^{-1}. The eluates were dried under a gentle stream of nitrogen. At this stage, an amount of 10 μl of 100 mg l^{-1} of pyren solution was added and the volume was adjusted to 100 μl with ethyl acetate. An aliquot of 1 μl was injected into the GC column.

To improve the recovery efficiency of extraction by increasing the ionic strength of sample, sodium chloride (5%) was added to all samples, causing the salting out effect and reducing the charge groups on the reversed-phase surface [16].

2.4. Optimization strategy

Eight variables that may affect the extraction efficiency and their possible interactions were studied. These variables are as follows: four eluting solvents, ethyl acetate, acetonitrile, methanol and hexane (factor A); type of sorbent, PLRP-S and C$_{18}$ (factor B); eluting solvent flow-rate, 0.2 and 1 ml min^{-1} (factor C); sample pH, 3 and 7 (factor D); sample volume, 200 and 400 ml (factor E); eluting solvent volume, 1 and 3 ml (factor F); addition of an organic modifier to the sample, no methanol added and 20% (v/v) methanol added (factor G); sample flow-rate, 3 and 6 ml min^{-1} (factor H). Because a four-level and seven two-level variables are to be optimized, the OA$_{32}$($4^1 \times 2^{28}$) matrix is employed to assign the variables considered and for the two-variable interactions which may occur. From the chemical point of view the interactions A×B, A×C, A×D, A×G, B×E, B×G, D×H, D×E and C×F should be considered. The assignment of the main-variables, possible two-variable interactions and their levels are given in Table 1.

The construction method of mixed-level OAD for a matrix with 32 experimental trials is similar to a matrix with 16 experimental trials, which was reported previously [5]. In order to construct a mixed-

level OAD and assignment of experiments, initially an original two-level OAD matrix and its assignment table must be constructed [4].

To estimate the effects of factors, after implementing the mixed-level OAD, the analysis of variance (ANOVA) technique is employed where both the purified sum of squares, SS′, and percentage contribution, PC (%), values for each factor can be computed. SS′ is defined as the sum of squares minus the variance due to error, while PC (%) is the relative contribution of SS′ for each factor, or error, to the total variance. The importance of a variable and/or an interaction can be estimated from the PC (%) values due to each significant factor. Furthermore, the PC (%) value due to error provides an estimate of the adequacy of the experiment [4,5,15].

3. Results and discussion

3.1. OAD study

SPE has been widely used to isolate the analytes of interest from the aquatic matrices in the last decade. To perform this technique either on-line or off-line, there are many stages, which an analyst has to take into consideration. Since the extraction process for a number of analytes occurs, more or less, in a single run, the efficiency of the recovery of each individual component differs from each other. This is due to the different chemical structures that every compound has and the low or high values of recoveries depend on the compatibility of each compound with the conditions of SPE. A detailed optimization of SPE would, therefore, help to adjust the applied conditions in a way to obtain the maximum recovery percentage for most of the constituents of the sample.

In this work, pesticides were selected due to their usage and identification from previous studies [17]. The selection of variables and their levels were based on published literature and previous knowledge [17,18]. The OA$_{32}$($4^1 \times 2^{28}$) matrix is given in Table 2. The constructing method of the matrix has been given in Ref. [5]. Each column represents a factor, which is an independent variable, and each row represents an experimental trial. The numbers at the intersections indicate the level settings that apply to

Table 1
The assignment of factors and levels of experiment using an $OA_{32}(4^1 \times 2^{28})$ matrix

Column no.																													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

Factor[a]

A	B	$(A \times B)_1$ **G×H**[b]	$(A \times B)_2$ **C×D**	$(A \times B)_3$ **F×E**	C	$(A \times C)_1$ **E×G**	$(A \times C)_2$ **B×D**	$(A \times C)_3$ **F×H**	$(A \times D)_2$ **B×C** **E×H**	$(A \times D)_3$	D	$(A \times D)_1$ **F×G**	E	(d) $(A \times E)_1$ **C×G**	D×H $(A \times E)_2$	(d) $(A \times E)_3$ **C×H**	B×E $(A \times F)_3$	(d) $(A \times F)_2$	(d) $(A \times F)_1$	F **D×G** **C×E**	$(A \times G)_1$ **B×H**	G **F×D**	$(A \times G)_3$	$(A \times G)_2$	H	B×G	D×E **$(A \times H)_1$**	F×C **$(A \times H)_2$** **$(A \times H)_3$**
I	PLRP-S				0.2							7		200						1		20			3			
II	C_{18}				1.0							3		400						3		0			6			
III																												
IV																												

[a] A=Eluting solvent (I: ethyl acetate; II: acetonitrile; III: hexane; IV: methanol); B=SPE sorbent; C=elution flow-rate (ml min^{-1}); D=sample pH; E=sample volume (ml); F=elution volume (ml); G=added methanol (%); H=sample flow-rate (ml min^{-1}); d=dummy cell.

[b] The interactions in bold can be neglected according to experience.

Table 2
$OA_{32}(4^1 \times 2^{28})$ matrix

Run no.	Column no.																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2
4	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1
5	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2
6	1	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1
7	1	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1
8	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2	2
9	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2
10	2	1	1	2	2	1	1	2	2	1	1	2	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2
11	2	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1
12	2	1	1	2	2	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2
13	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1
14	2	2	2	1	1	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	1	1	1	1	2	2
15	2	2	2	1	1	2	2	1	1	1	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2
16	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	1	1
17	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
18	3	1	2	1	2	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
19	3	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1
20	3	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2
21	3	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1
22	3	2	1	2	1	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2
23	3	2	1	2	1	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2
24	3	2	1	2	1	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1
25	4	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1
26	4	1	2	2	1	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2
27	4	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2
28	4	1	2	2	1	2	1	1	2	2	1	1	2	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1
29	4	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2
30	4	2	1	1	2	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1
31	4	2	1	1	2	2	1	1	2	1	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1
32	4	2	1	1	2	2	1	1	2	1	2	2	1	2	1	1	2	1	2	2	1	1	2	2	1	2	1	1	2

the factors for the experimental trials. After implementing 32 experimental trials that were pre-designed according to the above matrix, extraction recovery of the pesticides for each experimental trial was calculated. The results obtained are listed in Table 3. The average of the response for each factor at the different levels was calculated and is shown in Table 4. Evaluation of the recovery data by ANOVA allowed the determination of the variable and variable interactions that were significant to the SPE of these pesticides. From the assignment of experiments given in Table 1, it is obvious that four columns (columns 15, 17, 19 and 20) can be treated as dummies and used for calculating error variance since in which no actual factor and important two variable interaction can be assigned. The ANOVA results for the SPE recovery data will be discussed separately for each pesticide.

3.1.1. Diazinon

The analysis of variance for the recovery data of diazinon (Table 5) indicates that three main factors and seven interactions were significant to the recovery by SPE (at $P < 0.025$). No statistical differences are observed for factors C, D, E, F and H at $P > 0.1$, and interactions D×H and C×F at $P > 0.025$. From the percent contributions, it is observed that the most significant effect contributing to the re-

Table 3
Experimental results of the recovery extraction

Run no.	Recovery (%)			
	Atrazine	Diazinon	Ametryn	Fenthion
1	13.1	85.8	93.0	98.0
2	98.6	81.7	105.1	65.8
3	105.6	58.0	112.3	53.5
4	7.2	102.0	101.5	95.6
5	8.6	98.3	69.6	82.1
6	96.3	44.1	101.5	37.9
7	99.3	97.0	108.7	112.0
8	21.5	93.0	90.8	96.9
9	14.5	104.8	106.0	95.8
10	95.8	109.6	112.4	86.7
11	86.3	47.6	90.4	55.3
12	6.7	95.5	78.1	110.5
13	11.3	57.2	48.5	79.6
14	95.9	43.9	93.1	60.7
15	92.4	102.7	84.0	88.3
16	8.8	82.5	70.2	98.7
17	83.8	67.4	90.8	69.8
18	62.4	94.8	75.4	82.0
19	92.8	90.2	106.0	73.1
20	77.8	74.6	88.1	59.2
21	6.6	83.9	39.2	105.4
22	5.9	24.6	29.9	38.8
23	23.1	10.4	36.5	43.1
24	5.7	21.1	48.8	60.0
25	96.1	27.4	101.0	82.5
26	81.5	50.8	105.3	110.0
27	81.7	73.7	107.0	70.4
28	107.0	94.6	104.6	82.8
29	85.9	82.8	111.5	86.9
30	6.3	78.9	107.0	87.5
31	8.1	75.9	79.0	86.9
32	99.5	43.3	102.0	71.7

covery is interaction A×D (26.9%), and then A×B (15.5%), B×E (14.2%), A (13.6%), B (6.4%), D×E (4.82%), A×C (4.8%), A×G (3.9%), G (2.5%) and B×G (2.3%), accordingly. The percentage contribution due to error (unknown and uncontrolled factors) is low (3%). This means that no important variables and/or interactions have been omitted in this work.

In comparing r_1, r_2, r_3 and r_4 for factor A and r_1 and r_2 for the other factors in Table 4, it is clear that the optimum level for the significant variables are A_1, B_1 and G_1. In principle, for non-significant variables, any level values (for discrete variables) and/or any value between level 1 and level 2 (for continuous variables) are acceptable. However, when

an interaction that contains a non-significant variable is statistically significant, the choice of the optimum level values for these non-significant variables must be dependent on their interactions. In the other hand, the interactions A×B, A×C, A×D, A×G, B×E, D×E and B×G are statistically significant, therefore, the choice of the optimum experimental conditions of factors A, B and G, also, depend on their interactions. Evaluation of the optimum experimental conditions of the variables that contribute in the significant interactions was performed using the four-by-two and two-by-two tables. The method of construction of these tables is given in Refs. [4,5]. The four-by-two and two-by-two table for A×B, A×C, A×D, A×G, B×E, B×G and D×E interactions is shown in Table 6. The optimum combinations for these interactions, which give the maximum responses, are $A_2 \times B_1$, $A_1 \times C_2$, $A_2 \times D_1$, $A_1 \times G_1$, $B_1 \times E_2$, $D_1 \times E_1$ and $B_1 \times G_1$. Although, the combination of $A_2 \times B_1$ and $A_2 \times D_1$ would result in the maximum response, however, because of the recovery results with A_1 is near the A_2, the combination of $A_1 \times B_1$ and $A_1 \times D_1$ would nevertheless give a comparable result. Also, for the B×E interaction E_2 is optimum, and for the D×E interaction E_1 is optimum. Because PC% of the B×E interaction (14.2%) is greater than the value of the D×E interaction (PC% 4.8), the B×E interaction is more important than D×E and hence E_2 is preferable.

In summary, the results obtained from ANOVA indicate that by using a PLRP-S column, sample buffered at pH 7, sample volume of 400 ml, 20% methanol added, ethyl acetate elution solvent and 1 ml min⁻¹ elution flow-rate the maximum recovery is obtained. Furthermore, recovery is not affected when sample flow-rate is between 1 and 3 ml.

3.1.2. Atrazine

The recovery of atrazine was significantly affected by the main variables A, B and G and the interactions A×B, A×G and F×C at the $P < 0.025$ probability level (Table 7). Addition of an organic modifier to the sample is the most important factor (PC=45%). Addition of methanol to the sample reduced recovery of atrazine. Factors C, D, E, F and H and interactions A×C, A×D, B×E, B×G, D×H and D×E are not statistically significant at $P > 0.025$. Consideration of r_1, r_2, r_3 and r_4 for factor A and r_1

Table 4
Experimental results according to the $OA_{32}(4^1 \times 2^{28})$ matrix

Column no.																													
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Atrazine																												
r_1 56.3	69.4	41.8	59.3	52.5	53.9	58.1	54.1	55.0	56.5	56.8	55.5	56.3	56.8	54.8	58.4	53.6	57.1	55.9	57.1	56.0	40.9	28.3	48.3	62.1	52.1	58.8	59.8	50.2
r_2 51.5	42.2	69.8	52.3	59.1	57.7	53.5	57.5	56.6	55.1	54.9	56.1	55.3	54.8	56.8	53.2	58.0	54.5	55.7	54.5	55.6	70.8	83.3	63.4	49.5	59.5	52.8	51.8	61.4
r_3 44.8																												
r_4 70.8																												
Diazinon																												
r_1 82.5	78.7	69.1	76.4	62.5	71.0	69.3	74.8	67.2	71.8	85.0	70.3	67.0	72.7	70.9	74.4	71.6	61.6	70.5	72.1	70.8	75.9	76.2	75.6	72.0	70.6	76.0	65.8	74.5
r_2 80.5	65.0	74.5	67.3	81.1	72.6	74.3	68.9	76.5	71.9	58.6	73.3	76.6	70.9	72.7	69.2	72.0	82.0	73.2	71.5	72.8	67.7	67.5	68.1	71.7	73.0	67.6	77.8	69.1
r_3 58.4																												
r_4 65.9																												
Ametryn																												
r_1 97.8	98.6	84.5	91.7	80.0	86.8	87.6	82.5	87.3	88.1	91.5	86.8	84.9	86.5	85.9	89.3	86.1	90.6	87.3	87.3	86.1	84.0	81.5	87.5	87.6	87.7	91.3	87.3	86.7
r_2 85.3	76.3	90.3	83.1	94.8	88.0	87.3	92.3	87.6	86.8	83.4	88.0	89.9	88.4	89.0	85.5	88.7	84.2	87.5	87.6	88.8	90.9	93.3	87.3	87.2	87.2	83.5	87.5	88.1
r_3 64.3																												
r_4 102.2																												
Fenthion																												
r_1 80.2	80.7	77.6	78.6	77.1	79.3	72.1	77.8	77.4	84.3	84.1	78.8	81.7	80.2	78.5	84.1	79.3	71.9	78.3	78.7	78.3	86.0	84.3	82.1	76.5	78.1	84.9	75.9	82.8
r_2 84.5	77.3	80.4	79.4	80.9	78.6	85.9	80.2	80.5	73.7	73.8	79.1	76.3	77.8	79.4	73.9	78.7	86.1	79.6	79.2	79.6	72.0	73.7	76.0	81.4	79.9	73.1	82.1	75.2
r_3 66.4																												
r_4 84.8																												

Table 5
ANOVA including percent contribution for output responses in the $OA_{32}(4^1 \times 2^{28})$ matrix (data for extraction of diazinon)

Source	SS	df	MS	F [a]	SS′	PC (%)
Eluting solvent (A)	3233.8	3	1077.9	48.0[d]	3166.4	13.6
Sorbent (B)	1497.0	1	1497.0	66.7[e]	1475.0	6.3
Elution flow-rate (C)	21.3	1	21.3	0.95	–	–
Sample pH (D)	69.9	1	69.9	3.1	47.5	0.2
Sample volume (E)	24.7	1	24.7	1.1	2.2	–
Elution volume (F)	32.6	1	32.6	1.5	10.1	–
Added methanol (G)	601.2	1	601.2	26.8[g]	578.7	2.5
Sample flow-rate (H)	47.3	1	47.3	2.1	24.8	0.1
A×B	3658.3	3	1219.4	54.3[d]	3590.9	15.5
A×C	1175.3	3	391.8	17.4[f]	1108.0	4.8
A×D	6318.1	3	2106.0	93.8[b]	6250.7	27.0
A×G	983.3	3	327.8	14.6[h]	915.9	4.0
B×E	3310.9	1	3310.9	147.4[c]	3288.5	14.2
B×G	567.0	1	567.0	25.2[g]	544.5	2.3
D×H	210.6	1	210.6	9.4	188.2	0.8
D×E	1143.6	1	1143.6	50.9[e]	1121.2	4.8
C×F	237.1	1	237.1	10.5	214.6	0.9
Error	89.8	4	22.4	–	694.9	3.0
Total	23 222.3	31	–	–	23 222.3	100.0

[a] The critical F value is 24.3 ([d]$P<0.005$), 31.3 ([e]$P<0.005$), 16.7 ([f]$P<0.01$), 21.2 ([g]$P<0.01$), 9.99 ([h]$P<0.025$).

and r_2 for other factors in Table 4 and calculation of the four-by-two and two-by-two table (Table 8) for interactions A×B, A×G and C×F indicate that the optimum conditions are A_4, B_1, G_2, C_2 and F_2. Therefore, the optimum conditions are: methanol as the eluting solvent, PLRP-S sorbent, no added

Table 6
The four-by-two and two-by-two table for the analysis of the A×B, A×C, A×D, A×G, B×E, D×E and B×G interactions for diazinon

	Recovery (%)			
	A_1	A_2	A_3	A_4
B_1	81.9	89.4	81.8	61.6
B_2	83.1	71.6	35.0	70.2
C_1	77.5	78.9	67.7	60.0
C_2	87.5	82.1	49.1	71.9
D_1	89.4	99.9	68.3	82.5
D_2	75.6	61.1	48.4	49.4
G_1	94.8	85.0	55.0	69.8
G_2	70.2	76.0	61.8	62.0
	B_1	B_2	D_1	D_2
E_1	69.4	76.0	90.1	55.3
E_2	88.0	53.9	80.0	61.9
G_1	87.2	65.1	–	–
G_2	70.1	64.9	–	–

methanol to the sample, elution flow-rate of 1 ml min^{-1} and sample volume of 400 ml.

3.1.3. Ametryn

The ANOVA for ametryn demonstrated that three main effects and four interactions terms were significant (at $P<0.025$) to the recovery by SPE (Table 9). Of the significant variables, type of sorbent, elution solvent and added methanol influenced recovery of ametryn. The recovery was best at A_4 (or A_1), B_1 and G_2 (Table 4). The four-by-two and two-by-two table (Table 10) for interactions A×B, A×C, A×D and B×G, also, indicate that optimum conditions are A_4 (or A_1), B_1, C_2, D_1 and G_2. Hence, using methanol or ethyl acetate, PLRP-S, no added methanol to the sample, sample pH 7 and elution flow-rate of 1 ml min^{-1} result in the best recovery of ametryn. For others, non-significant variables (E, F and H), any level values and/or any value between level 1 and level 2 are acceptable.

3.1.4. Fenthion

Recovery of fenthion was significantly (at $P<0.025$) affected by factors A, B and G and most of the interactions (Table 11). At $P>0.025$ only the A×B interaction was not affected on the recovery.

Table 7
ANOVA including percent contribution for output responses in the $OA_{32}(4^1 \times 2^{28})$ matrix (data for extraction of atrazine)

Source	SS	df	MS	F[a]	SS′	PC (%)
Eluting solvent (A)	2917.9	3	972.6	16.1[c]	2736.2	5.1
Sorbent (B)	5932.3	1	5932.3	98.0[c]	5871.8	11.0
Elution flow-rate (C)	115.9	1	115.9	1.9	55.3	0.1
Sample pH (D)	2.5	1	2.5	4.1	–	–
Sample volume (E)	32.6	1	32.6	0.54	–	–
Elution volume (F)	1.2	1	1.2	0.02	–	–
Added methanol (G)	24 150.5	1	24 150.5	398.7[c]	24 090.0	45.0
Sample flow-rate (H)	443.3	1	443.3	7.3	382.7	0.7
A×B	7023.9	3	2341.3	38.7[d]	6842.2	12.8
A×C	282.9	3	94.3	1.6	101.2	0.19
A×D	51.4	3	17.1	0.3	–	–
A×G	10 221.0	3	3407.0	56.3[b]	10 039.3	18.8
B×E	53.8	1	53.8	0.89	–	–
B×G	293.4	1	293.4	4.8	232.9	0.43
D×H	213.7	1	213.7	3.5	153.2	0.3
D×E	516.0	1	516.0	8.5	455.4	0.8
C×F	1011.4	1	1011.4	16.7[f]	950.8	1.8
Error	242.3	4	60.6	–	1595.0	3.0
Total	53 506.1	31	–	–	53 506.1	100.0

[a] The critical F value is 56.2 ([b]$P<0.001$), 74.1 ([c]$P<0.001$), 24.3 ([d]$P<0.005$), 12.2 ([f]$P<0.025$).

The r values in the Table 4 and four-by-two and two-by-two table (Table 12) indicate that the optimum value for the factors are: A_4 (or A_2), B_1, G_1, $A_2 \times G_1$, $A_2 \times C_2$ (or $A_4 \times C_1$), $A_2 \times D_1$, $B_1 \times G_1$, $D_1 \times H_1$, $B_1 \times E_2$, $D_1 \times E_1$ and $F_2 \times C_2$. Here, like the case of diazinon, for the B×E interaction E_2 is the optimum, and for the D×E interaction E_1 is optimum. Because PC% of the B×E interaction (12.2%) is greater than the value of the D×E interaction (PC% 2.3), the B×E interaction is more important than D×E and hence E_2 is preferable. In summary, a sample pH of 7, PLRP-S, ACN eluting

Table 8
The four-by-two and two-by-two table for the analysis of the A×B, A×G and C×F interactions for atrazine

	Recovery (%)			
	A_1	A_2	A_3	A_4
B_1	56.1	50.8	79.2	91.6
B_2	56.4	52.1	10.3	50.0
G_1	12.6	10.3	46.1	44.4
G_2	100.0	92.6	43.5	97.1
	F_1	F_2		
C_1	57.0	50.8		
C_2	54.8	60.7		

solvent, 1 ml min^{-1} eluting flow-rate, 400-ml sample volume, 3-ml solvent volume, 20% methanol added and a sample flow-rate of 3 ml min^{-1} are optimum condition for the SPE of fenthion.

As a general conclusion for this part of study, it can be deduced that only main factors A, B and G are major constituents in this study and others are rather ineffective. The optimum condition for these pesticides is obtained when PLRP-S sorbent (B_1) and methanol (A_4) are being used. For diazinon, however, the use of ethyl acetate (A_1) and then acetonitrile (A_2) are preferred. The choice of PLRP-S, compared to C_{18}, for rather polar components has already been experienced by other researchers [19–21]. Sometimes the recoveries for the hydrophobic species (log $P_{ow}>4$) are being lost due to the adsorption of these compounds onto connecting tubes and containers and nothing to do with breakthrough. To avoid adsorption problem, the presence of an organic modifier, i.e., methanol, to the sample before percolation through the cartridge can effectively enhance the preconcentration recoveries of those pesticides [22]. Taking into consideration the obtained results, addition of methanol (G_1) is rather beneficial for obtaining higher recoveries for diazinon and fenthion. However, addition of this

Table 9
ANOVA including percent contribution for output responses in the $OA_{32}(4^1 \times 2^{28})$ matrix (data for extraction of ametryn)

Source	SS	df	MS	F^a	SS'	PC (%)
Eluting solvent (A)	6902.8	3	2301.0	69.6[b]	6803.7	38.4
Sorbent (B)	3976.1	1	3976.1	120.3[c]	3943.0	22.3
Elution flow-rate (C)	11.0	1	11.0	0.33	–	–
Sample pH (D)	11.0	1	11.0	0.33	–	–
Sample volume (E)	28.7	1	28.7	0.87	–	–
Elution volume (F)	59.7	1	59.7	1.8	26.6	0.15
Added methanol (G)	1124.6	1	1124.6	34.0[e]	1091.5	6.2
Sample flow-rate (H)	2.1	1	2.1	–	–	–
A×B	2616.2	3	872.1	26.4[d]	2517.0	14.2
A×C	778.8	3	259.6	7.8[g]	679.6	3.8
A×D	743.6	3	247.9	7.5[g]	644.5	3.6
A×G	378.8	3	126.3	3.8	279.7	1.6
B×E	327.0	1	327.0	9.9	294.0	1.7
B×G	487.5	1	487.5	14.7[f]	454.4	2.6
D×H	113.6	1	113.6	3.4	80.6	0.45
D×E	0.23	1	0.23	–	–	–
C×F	16.4	1	16.4	0.50	–	–
Error	132.2	4	33.0	–	895.5	5.0
Total	17 710.2	31	–	–	17 710.2	100.0

[a] The critical F value is 56.2 ([b]$P<0.001$), 74.1 ([c]$P<0.001$), 24.3 ([d]$P<0.005$), 31.3 ([e]$P<0.005$), 12.2 ([f]$P<0.025$), 6.6 ([g]$P<0.05$).

organic modifier lowers the recoveries of atrazine and ametryn. In the other hand, diazinon and fenthion, log $P_{ow}>3.8$, are more hydrophobic than atrazine and ametryn, log $P_{ow} \sim 2.5$, and this is in agreement with our results.

Our statistical approach showed that the sample pH on its own has no influence on recoveries. This confirms the previously reported data [23]. The

sample pH is quite important when one deals with ionic species such as acidic herbicides and phenols. In such cases, when the media is acidic, the ionic species are neutralised and are adsorbed on the non-polar sorbent more efficiently. Although, the studied pesticides in this work exhibit some polar behaviour but they are considered as non-ionic species. The rest of the main variables, i.e., C, E, F and H, can be regarded as non-significant variables. As explained before, when an interaction contains a non-significant variable is statistically significant, the choice of the optimum level values for these non-significant variables must be considered.

3.2. Real sample analysis

The final optimized condition for the SPE of these four pesticides from different classes was applied to the SPE of river Karoun water. Taking into consideration the retention data from the real-sample and spiked-sample chromatograms, atrazine and ametryn were tentatively identified and quantified at the 0.7 and 0.9 μg l^{-1} levels, respectively. These results are in agreement with those already measured employing GC-FID [24] and GC-MS-AED [25]. Table 13

Table 10
The four-by-two and two-by-two table for the analysis of the A×B, A×C, A×D and B×G interaction for ametryn

	Recovery (%)			
	A_1	A_2	A_3	A_4
B_1	103.0	96.7	90.1	104.5
B_2	92.7	74.0	38.6	99.9
C_1	92.3	90.0	58.8	106.2
C_2	103.3	80.7	69.9	98.2
D_1	99.4	93.1	65.8	107.5
D_2	96.2	77.5	62.9	96.8
	B_1	B_2		
G_1	96.5	66.4		
G_2	100.6	86.1		

Table 11
ANOVA including percent contribution for output responses in the $OA_{32}(4^1 \times 2^{28})$ matrix (data for extraction of fenthion)

Source	SS	df	MS	F[a]	SS'	PC (%)
Eluting solvent (A)	1787.3	3	595.8	91.9[b]	1767.8	13.5
Sorbent (B)	92.82	1	92.82	14.3[e]	86.3	0.66
Eluting flow-rate (C)	4.1	1	4.1	0.64	–	–
Sample pH (D)	0.75	1	0.75	0.12	–	–
Sample volume (E)	44.9	1	44.9	6.9	38.4	0.3
Elution volume (F)	13.9	1	13.9	2.1	7.4	–
Added methanol (G)	908.4	1	908.4	140.1[c]	901.9	6.9
Sample flow-rate (H)	25.7	1	25.7	4.0	19.2	0.15
A×B	183.7	3	61.2	9.4	164.3	1.3
A×C	1635.1	3	545.0	84.1[b]	1615.7	12.4
A×D	1970.7	3	656.9	101.3[b]	1951.2	14.9
A×G	2054.3	3	684.8	105.6[b]	2034.9	15.6
B×E	1600.3	1	1600.3	246.8[c]	1593.9	12.2
B×G	1117.5	1	1117.5	172.3[c]	1111.0	8.5
D×H	837.4	1	837.4	129.1[c]	830.9	6.4
D×E	313.1	1	313.1	48.3[d]	306.6	2.3
C×F	453.8	1	453.8	70.0[d]	447.3	3.4
Error	25.9	4	6.48	–	193.0	1.5
Total	13 069.9	31	–	–	13 069.9	100.0

[a] The critical F value is 56.2 ([b]$P<0.001$), 74.1 ([c]$P<0.001$), 24.3 ([d]$P<0.005$), 31.3 ([e]$P<0.005$).

shows the recovery percentage, limits of detection and concentration of those that were quantified in the water sample.

Table 12
The four-by-two and two-by-two table for the analysis of the A×G, A×C, A×D, B×E, D×E, B×G, D×H and C×F interactions for fenthion

	Recovery (%)			
	A_1	A_2	A_3	A_4
G_1	92.7	96.2	59.3	88.7
G_2	67.3	72.8	73.6	81.0
C_1	71.0	80.7	74.0	91.7
C_2	89.5	88.2	58.9	78.0
D_1	93.1	92.4	69.1	81.9
D_2	67.3	76.5	63.7	87.8
	B_1	B_2	D_1	D_2
E_1	74.8	85.5	91.2	69.1
E_2	86.6	69.0	77.1	78.6
G_1	91.9	76.7	–	–
G_2	69.5	77.9	–	–
H_1	–	–	93.0	72.5
H_2	–	–	75.3	75.2
	F_1	F_2		
C_1	84.6	74.1	–	–
C_2	72.1	85.2	–	–

4. Conclusion

The $OA_{32}(4^1 \times 2^{28})$ matrix which has been designed, in here, clearly demonstrates the effects of different important parameters influencing the extraction efficiency in detail and their interactions. Many aspects of the results confirm the previously reported experimental data. Using the OAD method, not only the optimum extraction condition for different pesticides could be achieved, but a great deal of information about the effects of each factor on the recovery is obtained while the minimum number of experiments is performed. A major and clear point from this study is that a general SPE procedure can

Table 13
Limits of detection (LOD), recoveries and concentrations of the studied pesticides in Karoun river water ($N=3$)

Compound	LOD ($\mu g \, l^{-1}$)	Recovery		Concentration	
		%	SD	$\mu g \, l^{-1}$	SD
Atrazine	0.08	96	0.73	0.90 ± 0.04	2.8
Diazinon	0.04	92	1.1	ND[a]	–
Ametryn	0.03	95	1.7	0.70 ± 0.03	2.6
Fenthion	0.10	92	1.5	ND	–

[a] Not detected.

be designed for isolation and preconcentration of various pesticides, each representing a different class of pesticides, from water samples.

Acknowledgements

References

[1] G. Font, J. Manes, J.C. Molto, Y. Pico, J. Chromatogr. 642 (1993) 135.

[2] U.A.Th. Brinkman, Th. Hankemeier, J.J. Vreuls, Chem. Anal. (Warsaw) 40 (1995) 495.

[3] M. Moors, D.L. Massart, R.D. McDowall, Pure Appl. Chem. 66 (1994) 277.

[4] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1659.

[5] W.G. Lan, K.K. Chee, M.K. Wong, H.K. Lee, Y.M. Sin, Analyst 120 (1995) 281.

[6] R.E. Hannah, V.L. Cuningham, J.P. McGough, in: I.H. Suffet, M. Malaiyandi (Eds.), Organic Pollutants in Water, ACS Advances in Chemistry Series, Vol. 214, ACS, Washington, DC, 1987, p. 359.

[7] M.J.M. Wells, J.M. Micheal, J. Chromatogr. Sci. 25 (1987) 345.

[8] M.J.M. Wells, D.D. Riemer, M.C. Wells-Knecht, J. Chromatogr. A 659 (1994) 337.

[9] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok, Y.H. Poh, J. Chromatogr. A 677 (1994) 255.

[10] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1669.

[11] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok, Anal. Chim. Acta 289 (1994) 371.

[12] G. Taguchi, Systems of Experimental Design, Vol. 1–2, Kraus, New York, 1987.

[13] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Talanta 41 (1994) 1917.

[14] W.G. Lan, K.K. Chee, M.K. Wong, Y.M. Sin, Analyst 120 (1995) 273.

[15] P.J. Ross, Taguchi Techniques For Quality Engineering, McGraw Hill, New York, 1988.

[16] S.A. Schuette, R.G. Smith, L.R. Holden, A. Graham, Anal. Chim. Acta 236 (1990) 141.

[17] H. Bagheri, L. Van Stee, P.E.G. Leonards, J.J. Verules, U.A.Th. Brinkman, submitted.

[18] H. Bagheri, R.M. Marce Recasens, J. Slobodnik, R.T. Ghijsen, U.A.Th. Brinkman, Chromatographia 37 (1993) 159.

[19] J. Hodgeson, J. Collins, W. Bashe, J. Chromatogr. 659 (1994) 395.

[20] M. Hennion, V. Coquart, S. Guenu, C. Sella, J. Chromatogr. 712 (1995) 287.

[21] C. Molina, P. Grasso, E. Benfenati, D. Barcelo, J. Chromatogr. 737 (1996) 47.

[22] G.R. van der Hoff, F. Pelusio, U.A.Th. Brinkman, R.A. Baumann, P. van Zoonen, J. Chromatogr. 719 (1996) 59.

[23] J.M. Vinuesa, J.C.M. Cortes, C.I. Canas, G.F. Perez, J. Chromatogr. 472 (1989) 365.

[24] M. Chitsazan, Solid Phase Extraction and Chromatographic Methods For Identification and Quantification of Organic Micropollutants in River Karoun Water, Sharif University of Technology, Tehran, Iran, 1998, M.Sc. Thesis.

[25] H. Bagheri, M. Saraji, U.A.Th. Brinkman, Environmental Application of GC-AED, in: D. Barcelo, (Eds.), Environmental Analysis Techniques, Applications and Quality Assurance, Elsevier, The Netherlands, 2000.