

Optimization of microwave-assisted extraction and supercritical fluid extraction of carbamate pesticides in soil by experimental design methodology

Lei Sun, Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Abstract

Orthogonal array design (OAD) was applied for the first time to optimize microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) conditions for the analysis of four carbamates (propoxur, propham, methiocarb, chlorpropham) from soil. The theory and methodology of a new OA_{16} (4^4) matrix derived from a OA_{16} (2^{15}) matrix were developed during the MAE optimization. An analysis of variance technique was employed as the data analysis strategy in this study. Determinations of analytes were completed using high-performance liquid chromatography (HPLC) with UV detection. Four carbamates were successfully extracted from soil with recoveries ranging from 85 to 105% with good reproducibility (~4.9% RSD) under the optimum MAE conditions: 30 ml methanol, 80 °C extraction temperature, and 6-min microwave heating. An OA_8 (2^7) matrix was employed for the SFE optimization. The average recoveries and RSD of the analytes from spiked soil by SFE were 92 and 5.5%, respectively except for propham ($66.3 \pm 7.9\%$), under the following conditions: heating for 30 min at 60 °C under supercritical CO_2 at 300 kg/cm² modified with 10% (v/v) methanol. The composition of the supercritical fluid was demonstrated to be a crucial factor in the extraction. The addition of a small volume (10%) of methanol to CO_2 greatly enhanced the recoveries of carbamates. A comparison of MAE with SFE was also conducted. The results indicated that >85% average recoveries were obtained by both optimized extraction techniques, and slightly higher recoveries of three carbamates (propoxur, propham and methiocarb) were achieved using MAE. SFE showed slightly higher recovery for chlorpropham (93 vs. 87% for MAE). The effects of time-aged soil on the extraction of analytes were examined and the results obtained by both methods were also compared.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Soil; Experimental design; Orthogonal array design; Microwave-assisted extraction; Supercritical fluid extraction; Extraction methods; Pesticides; Carbamates

1. Introduction

Carbamate pesticides, as constituents of the organochlorine compounds, are gaining importance in the field of pest control because of their high

efficiency as insecticides and nematicides, their low bioaccumulation potentials and their relatively low mammalian toxicities. However, since they are acetylcholinesterase inhibitors, they are considered hazardous to the environment and human health [1–3]. For these reasons carbamates are on the priority list released by the US Environmental Protection Agency [4]. Evaluation and monitoring of trace levels of these compounds in soil are impera-

*Corresponding author. Tel.: +65-6874-2995; fax: +65-6779-1691.

E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

tive because the widespread use of carbamates in agriculture leads to an increase in the presence of their residues in environmental matrices, especially soil.

Typical characteristics of carbamate pesticides are high polarity and solubility in water and thermal instability. Therefore, direct analysis by gas chromatography (GC) is difficult because the compounds are rapidly decomposed to the corresponding phenols and amines during the determination. Liquid chromatography (LC) has been applied routinely in the analysis of carbamates due to its suitability for thermally labile and polar pesticides. The coupling of LC and mass spectrometry (MS) is a powerful technique for the determination of carbamate residues and other polar and thermally labile compounds especially since the technique provides excellent sensitivity and selectivity [5–7].

The accuracy and precision of an analytical method are directly dependent on sample treatment. To obtain the cleanest samples and to avoid pesticide degradation during the extraction from a sample matrix, an adequate preconcentration technique with optimum extraction conditions is crucial prior to the final determination.

Since conventional liquid–solid extraction techniques, such as Soxhlet extraction, sonication and mechanical shaking, are laborious, time-consuming and need large volumes of toxic organic solvents, much attention is being paid to the development of more efficient environmentally friendlier techniques for the rapid analytical-scale extraction from solid matrices, such as supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE). SFE of environmental contaminants has been reported from various solid matrices. The unique properties of supercritical fluids (SCFs) have made SFE a practical alternative to traditional liquid solvent extraction techniques [8,9]. For example, supercritical CO₂, which has been used for extraction is a nontoxic, nonflammable, and generally considered to be a comparatively environmentally friendly solvent. In addition, because CO₂ has a very moderate critical temperature (31.3 °C) and chemical inertness, SFE is specially recommended for thermally labile compounds such as carbamate pesticides. Surprisingly, there have been only a few applications reported using SFE for the analysis of carbamate pesticides.

Recently, Jeong and Chesney used SFE to extract three *N*-methylcarbamate pesticides (carbaryl, aldicarb, and carbofuran) from spiked filter paper and silica gel matrices. Recoveries from such spiked matrices ranged between 13 and 100%, depending on the analyte and the matrix components [10]. The use of statistical techniques on SFE optimization has provided the analyst with a quick, relatively accurate optimization technique that would otherwise have been costly in both time and materials. Except for Stuart et al. who optimized SFE conditions using analysis of variance (ANOVA) techniques to successfully extract three carbamates (carbaryl, aldicarb and pirimicarb) from soil [11], there have been few investigations focusing on optimization of SFE parameters for the extraction of carbamate pesticides. Considering the above, it is of interest to use SFE to extract carbamate pesticides from solid matrices and develop a strategy to optimize the process.

MAE has been successfully applied to the simultaneous extraction of toxic organic contaminants from different solid matrices, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols and pesticides [12–24]. Ganzler and co-workers were the first to report the use of microwave energy to irradiate solid matrices such as soil, seeds, foods, and feeds in the presence of extracting solvents with high dipole moments [12,21]. Hasty and Revesz extracted petroleum hydrocarbons from soil [22]. Lopez-Avila and co-workers used microwave energy to extract several groups of pollutants such as PAHs, PCBs, pesticides, phenolics and base/neutral compounds in soils and sediments [23,24]. In all these studies, MAE proved to be more efficient and the sample preparation time and solvent consumption volumes are all largely reduced in comparison to the many hours and hundreds of milliliters of organic solvents needed for Soxhlet and sonication methods. Although MAE has many advantages, little work has been reported on the MAE of carbamates. This may be because degradation of these thermally labile compounds occurs easily during the microwave heating.

In order to obtain optimum MAE and SFE conditions for carbamate extractions, we have employed the orthogonal array design (OAD) procedure. OAD as a chemometric method was introduced into the field of analytical chemistry in 1989 by Ole and

Yankovich, who employed this design to optimize the operating conditions of GC analysis [25]. Since then, there have been a considerable number of reports on the optimization of analytical procedures using OAD [25–38]. This chemometric approach has the advantages of both sequential optimization procedures (simplex optimization) and simultaneous optimization procedures (factorial designs). The details of the theory and methodology of the OAD approach for the optimization of analytical procedures have been described [31–37]. Although the two-level OAD can be used to study both the main factors and the interactions between them as well, the two-level OAD is based on the assumption of a linear response surface. This is not always the most reasonable assumption. When there is a significant curvature in the response surface, the multilevel (more than two) OAD has to be followed. The four-level OAD is a direct extension of the two-level OAD with the added capability of providing information about the non-linearity of the main factors in the response surface. However, the method as described in a work by Lan et al. ignored the interaction terms in the four-level design [32]. In this work, a new four-level OAD procedure is derived which can provide information not only of the main factors, but of some interactions between them as well.

In our previous work, MAE was applied for the first time to study carbamates [39]. We found that significant thermal degradation of the target analytes

occurred and subsequently low recoveries were obtained from soil under the applied extraction conditions. In order to improve the extraction recoveries of carbamate pesticides (propoxur, propham, methiocarb, chlorpropham, see Fig. 1) from soil using MAE and SFE techniques, the present work is firstly focused on method optimization of both techniques by OAD. Secondly, the application of both optimized techniques for the extraction of carbamate pesticides in soil samples is described. Finally, the comparative recoveries of four carbamates by both MAE and SFE under their respective optimum conditions are discussed.

2. Experimental

2.1. Reagents and soil preparation

All solvents used in this study were either pesticide-grade or HPLC-grade and were obtained from Fischer (Fair Lawn, NJ, USA). The water used was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The four carbamates studied were propoxur (purity 99%), methiocarb (purity 99%), propham (purity 99.5%) and chlorpropham (purity 99.5%). They were purchased from ChemService (West Chester, PA, USA). The structures of the tested carbamates are shown in Fig. 1. Stock solutions containing each compound (1000 $\mu\text{g}/\text{ml}$) were prepared in methanol and diluted with methanol to obtain working solutions at various concentrations. They were stored at 4 $^{\circ}\text{C}$.

Blank soils, collected from local sites were air-dried, ground and sieved through a 60-mesh sieve. To prepare carbamate-free soils, soil samples were immersed sequentially in methanol, acetone, dichloromethane and *n*-hexane for at least 24 h each. Finally, it was determined that there were no detectable levels of the target analytes in the soil samples before spiking.

Spiked samples were prepared by adding an appropriate volume of spiking solution to homogenize the soils prepared above. After being air-dried overnight, the spiked soil samples were set aside 10–14 days prior to soil analysis.

Aged spiked soil samples were obtained by storing

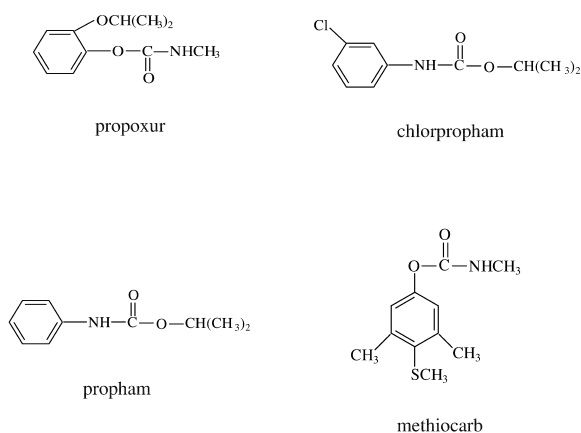


Fig. 1. Names and structural formulae of carbamates used in this study.

the above spiked soil in bottles in a dry, dark location for 60 days. It was assumed that the contaminants were uniformly distributed in the sample and that, because the soil still retained residual moisture throughout the storage period, any analyte–matrix interactions would have occurred, over the weathering period, to a similar extent to those in real contaminated soil with similar properties.

2.2. Instrumentation

Microwave-assisted extraction was carried out using a MarsX (1200-W) laboratory microwave extraction system (CEM, Matthews, NC, USA) equipped with a solvent detector. The instrument is able to extract concurrently 14 solid samples in PTFE extraction vessels under identical extraction conditions. An inboard temperature control system is installed for monitoring and controlling temperature conditions inside the extraction vessels.

SFE in the dynamic mode was carried out using a PU-980 HPLC pump (Jasco, Tokyo, Japan). A Trace Series 2000 (ThermoQuest, Rodano, Italy) GC oven was used to produce the required critical temperature of CO₂. A 10-ml stainless steel sample cell (Jasco) was installed in the GC oven. Methanol was added to the CO₂ at intervals with an additional PU-980 HPLC pump.

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump, a Rheodyne (Cotati, CA, USA) 7010 injector equipped with a 20- μ l loop, a Shimadzu SPD-6AV UV–Vis detector and a Shimadzu C-R6A integrator. In this study, a Phenomenex (Torrance, CA, USA) ODS 150 \times 3.2 mm column was used. The detection wavelength was 225 nm. The mobile phase was acetonitrile–water (40:60) at a flow-rate of 0.8 ml/min.

2.3. Microwave-assisted extraction procedure

In this study, 2 g of spiked soil samples was accurately weighed out and quantitatively transferred to the MarsX extraction vessels. According to a pre-designed experimental trial, the respective volumes of the extracting solvents were added into the vessels and extraction carried out under different MAE conditions. After extraction, the vessels were cooled down to room temperature before opening.

Sample extracts were further clarified by centrifugation at 4000 rpm for 15 min to separate out the fine particulates. The supernatant was then transferred to a round-bottomed flask and evaporated to dryness in a rotary evaporator. Finally, 1 ml of methanol was added to dissolve the residue and 10 μ l of the solution was directly analyzed by HPLC.

2.4. Supercritical fluid extraction procedure

Four grams of soil was loaded into the extraction cell. CO₂, pure or premixed with 10% methanol, was used as the extraction fluid. The extraction process started after the extraction vessel attained working conditions, based on pre-designed experimental trials. After equilibration for 10 min, the supercritical fluid was passed through the extraction cell containing the sample. The flow-rate of the supercritical fluid was 1–2 ml/min measured at the pump. After extraction, extracts were collected by bubbling the vented CO₂ through methanol and finally concentrated to 2 ml using a rotary evaporator before HPLC analysis.

3. Results and discussion

3.1. Optimization strategy

A two-level orthogonal array design, denoted by OA_{*s*+1} (2^{*s*}), is based on an (*S*+1) \times *S* (row \times column) matrix, whose column number corresponds to the number of factors and row number to the number of experimental trials. The intersections between the columns and rows indicate the level settings that apply to those factors for the experimental trials [32].

An OA₁₆ (4⁴) matrix with 16 treatments and four main factors (A, B, C, D) are derived from an OA₁₆ (2¹⁵) matrix based on methodologies derived in Ref. [40]. A1, A2, A3, B1, B2, B3, C1, C2, C3 and D1, D2, D3 are used to represent three degrees of freedom for factors A, B, C and D, respectively. Their assignment in an OA₁₆ (2¹⁵) matrix is shown in Table 1. In addition, columns 13, 14, 15 are assigned to three two-variable interactions AB, AC and BC which, as indicated by their names, are interactions between A and B, between A and C, and between B and C, respectively. Only two-factor

Table 1
The OA₁₆ (2¹⁵) matrix used to process OA₁₆ (4⁴) matrix

Experiment no.	Column no.														
	1 A1	2 A2	3 B1	4 B2	5 C1	6 D1	7 C2	8 D2	9 A3	10 B3	11 C3	12 D3	13 AB	14 AC	15 BC
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	1
3	-1	1	-1	-1	1	1	1	-1	-1	1	1	-1	1	-1	-1
4	1	1	-1	-1	-1	-1	1	1	1	1	-1	-1	-1	1	-1
5	-1	-1	1	-1	-1	1	1	1	1	-1	-1	1	1	-1	-1
6	1	-1	1	-1	1	-1	1	-1	-1	-1	1	1	-1	1	-1
7	-1	1	1	-1	1	-1	-1	1	-1	-1	-1	-1	1	1	1
8	1	1	1	-1	-1	1	-1	-1	1	-1	1	-1	-1	-1	1
9	-1	-1	-1	1	1	-1	1	1	1	-1	1	-1	-1	-1	1
10	1	-1	-1	1	-1	1	1	-1	-1	-1	-1	-1	1	1	1
11	-1	1	-1	1	-1	1	-1	1	-1	-1	1	1	-1	1	-1
12	1	1	-1	1	1	-1	-1	-1	1	-1	-1	1	1	-1	-1
13	-1	-1	1	1	1	1	-1	-1	1	1	-1	-1	-1	1	-1
14	1	-1	1	1	-1	-1	-1	1	-1	1	1	-1	1	-1	-1
15	-1	1	1	1	-1	-1	1	-1	-1	1	-1	1	-1	-1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

interactions are considered since experience tells that interactions among three or more factors will not be significant. The rest of the three possible two-factor interactions, AD, BD and CD are not listed because of the same argument.

With those assignments, the method to derive a four-level OA from a two-level OA is straightforward: levels are horizontally combined in two columns in a two-level OA to index one out of the four levels for each main factor. There will be four possible combinations, (-1, -1), (1, -1), (-1, 1) and (1, 1), which are assigned to levels I, II, III and IV, respectively. The columns used to decide the levels for factors A, B, C, D are combinations of (A1, A2), (B1, B2), (C1, C2), and (D1, D2), respectively. The resulting experiment level assignments to each factor are shown in Table 2.

On the basis of the above consideration, a new OA₁₆ (4⁴) matrix, which is used for the experimental design of the MAE optimization, is only established from columns A1 to D1 (1 to 8) in Table 1. The rest of the columns in the table are employed for data analysis.

3.2. Experimental design for MASE optimization

With MAE, extraction solvents, extraction tem-

perature, time and solvent volume appeared to be the major parameters affecting extraction efficiency. In this study, the main factors considered in the MAE

Table 2
A design process from OA₁₆ (2¹⁵) matrix to OA₁₆ (4⁴) matrix

Experiment no.	A	B	C	D
1	I ^a	I	I ^b	I ^c
2	II ^a	I	II	IV
3	III ^a	I	IV	II
4	IV ^a	I	III	III
5	I	II	III	IV
6	II	II	IV	I
7	III	II	II	III
8	IV	II	I	II
9	I	III	IV	III
10	II	III	III	II
11	III	III	I	IV
12	IV	III	II	I
13	I	IV	II	II
14	II	IV	I	III
15	III	IV	III	I
16	IV	IV	IV	IV

^a I, II, III, IV: four levels of each factor; they can be represented by (-1, -1), (1, -1), (-1, 1), (1, 1), respectively.

^b Four levels (I, II, III, IV) are obtained by combining columns C1 and C2.

^c Four levels (I, II, III, IV) are obtained by combining columns D1 and D2.

Table 3
Main factors and their level settings considered in the MAE optimization process

Level setting values	Main factors			
	Extraction solvents (A)	Extraction temperature (B, °C)	Extraction time (C, min)	Volume of solvents (D, ml)
I	Methanol	40	4	10
II	Dichloromethane	60	6	20
III	Acetone–hexane (1:1, v/v)	80	8	30
IV	Acetone	100	10	40

optimization process are displayed in Table 3. The variables used in this optimization are: (1) extraction solvents (factor A); (2) extraction temperature (factor B); (3) extraction time (factor C); (4) volume of extraction solvents (factor D). The level setting values of the main variables (A, B, C, D) are also shown in the table. For example, the four types of extracting solvents considered include: methanol (I), acetone (II), acetone–hexane (1:1, v/v, III) and dichloromethane (IV). Table 4 shows the experimental design of the MAE optimization conditions and the results obtained from each experimental trial. From this table, we can see that a new OA_{16} (4^4) matrix with 16 treatments derived from a two-level OA_{16} (2^{15}) matrix (mentioned in detail above) is employed to assign the variables and the corresponding level settings considered. We can also see that in different experimental trials, the variables were varied with the level setting values. Average recovery (AR) was calculated from the sum of percentage recoveries from the soil that was spiked with the four carbamates. It can be used as a response function because it can take into consideration the effect of changes in the variables on the extraction efficiency of this technique. After implementing the 16 experimental trials, the corresponding average recoveries (AR) from each experimental trial for the variables set were calculated and then tabulated (Table 4). In addition, the average responses of each factor at different levels (I, II, III, IV) are also given in the table.

3.3. Data analysis strategy

The matrix OA_{16} (4^4) is only useful for the experiments. Data processing is done through the original OA_{16} (2^{15}) matrix from which OA_{16} (4^4) is

derived. The normal two-level ANOVA techniques are employed and the results are shown in Table 5. A minor modification is that the SS for each main factor is the sum of SS from three columns since each of them has three degrees of freedom and occupies three columns. Specifically, SS for the factor A is the sum of SS of A1, A2 and A3 for example. Since no dummy columns are assigned, error estimation is done by experiment repetition.

Table 4
 OA_{16} (4^4) matrix as the experimental design of the MAE optimization for the extraction of carbamates

Experimental trials	Column no.				Average recovery (%)
	A ^a	B ^a	C ^a	D ^a	
1	I ^b	I	I	I	64.0
2	II ^b	I	II	IV	80.2
3	III ^b	I	IV	II	62.2
4	IV ^b	I	III	III	67.4
5	I	II	III	IV	91.1
6	II	II	IV	I	82.4
7	III	II	II	III	77.6
8	IV	II	I	II	72.9
9	I	III	IV	III	91.1
10	II	III	III	II	93.8
11	III	III	I	IV	85.3
12	IV	III	II	I	78.5
13	I	IV	II	II	59.4
14	II	IV	I	III	24.0
15	III	IV	III	I	8.3
16	IV	IV	IV	IV	9.3
I ^c	76.4	68.5	61.6	58.3	
II ^c	70.1	81.0	73.9	65.0	
III ^c	58.4	87.2	65.2	72.1	
IV ^c	57.0	25.3	61.3	66.5	

^a Four main factors; they can be represented by (−1, −1), (1, −1), (−1, 1), (1, 1), respectively.

^b Four levels for each main factor; they can be represented by (−1, −1), (1, −1), (−1, 1), (1, 1), respectively.

^c Average response of each level.

Table 5
ANOVA table including percent contribution for the percentage recovery in the OA₁₆ (2¹⁵) matrix

Parameter	A	B	C	D	AB	AC	BC	Error
SS	4639	37 998	1492	733	433	171	841	7054
df	3	3	3	3	1	1	1	48
MS	1546	12 666	497	244	433	171	841	147
F-value	10.52	86.19	3.38	1.66	2.95	1.16	5.72	–
P-value ^a	<0.05*	<0.05*	<0.05*	–	–	–	<0.05*	–

SS, sum of squares; df, degrees of freedom.

^a The critical F-value is 2.8 (* $P < 0.05$).

The SS of error is the residue of the total SS after subtracting all the SS of the items [40].

Based on the ANOVA results in Table 5, factor A (extraction solvent), factor B (extraction temperature), factor C (extraction time) and the two-variable interaction B×C (interaction between extraction temperature and extraction time) are statistically significant at $P < 0.05$. Both A×B (interaction between extraction solvents and temperature) and A×C (interaction between extraction solvents and time) are not statistically significant. It is reasonable to neglect these insignificant factors during the optimization.

Since the significant factor A is independent of factors B and C, a further experimental design was established to obtain optimum conditions of factor A. The factor A varies among four levels while factors B and C are arbitrary constants (60 °C and 8 min, respectively). The volume of extraction solvent (factor D) has no significant effect on MAE and it is set at 30 ml throughout. Results in Table 6 clearly show that the highest average recovery of targets was obtained in methanol. Therefore, the optimum extraction solvent (factor A) is methanol.

Since the interaction between extraction temperature and time (B×C) is another statistically significant variable ($P < 0.05$), the choice of the optimum

experimental conditions for factors B and C must depend on their interaction. Methanol is used as extraction solvent to extract the four targets from soil under the MAE conditions with different extraction temperatures and time. The experimental design and results are displayed in a 4×4 table (Table 7) where the B×C interaction is evaluated. It is clear that for AR, the combination of B₃ and C₂ would result in the maximum response, i.e. 95.9.

Thus, the optimum MAE conditions for the extraction of carbamates from soil samples were as follows: 30 ml methanol as extraction solvent was added into the MAE vessel, which contained 2.0 g of spiked soil sample, and then extraction was carried out at 80 °C with duration time of 6 min under microwave heating. It should be noted that the microwave power was not optimized as it depends

Table 7
4×4 table for the analysis of the B×C interaction

	Average recovery (%)			
	B ₁	B ₂	B ₃	B ₄
C ₁	64.9	82.0	85.5	60.0
C ₂	82.8	88.4	95.9	57.5
C ₃	83.9	89.9	92.9	52.2
C ₄	85.8	89.5	92.4	47.2

Table 6
Recoveries of the four carbamates extracted from soil under MAE conditions of heating for 8 min at 60 °C in 30 ml of different extraction solvents

Extraction solvent (A)	Recoveries (%) ± RSD (%), n=4				Average recovery (%)
	Propoxur	Propham	Methiocarb	Chlorpropham	
Methanol	99.05 ± 5.6	76.28 ± 6.9	102.3 ± 3.1	81.80 ± 6.4	89.9
Acetone–hexane (1:1)	80.38 ± 4.8	78.45 ± 7.3	83.70 ± 5.6	78.55 ± 7.5	80.3
Dichloromethane	79.65 ± 7.4	70.40 ± 5.4	80.00 ± 4.6	77.54 ± 5.9	76.9
Ethyl acetate	68.99 ± 4.5	68.03 ± 9.8	65.24 ± 9.3	79.52 ± 8.2	70.5

on the number of samples to be extracted in one run; 95% or greater recovery can be obtained under such optimized conditions. Evidence is presented in Fig.

2, which shows a standard chromatogram (Fig. 2a) and a typical chromatogram of a mixture of the four carbamates under the optimum MAE conditions

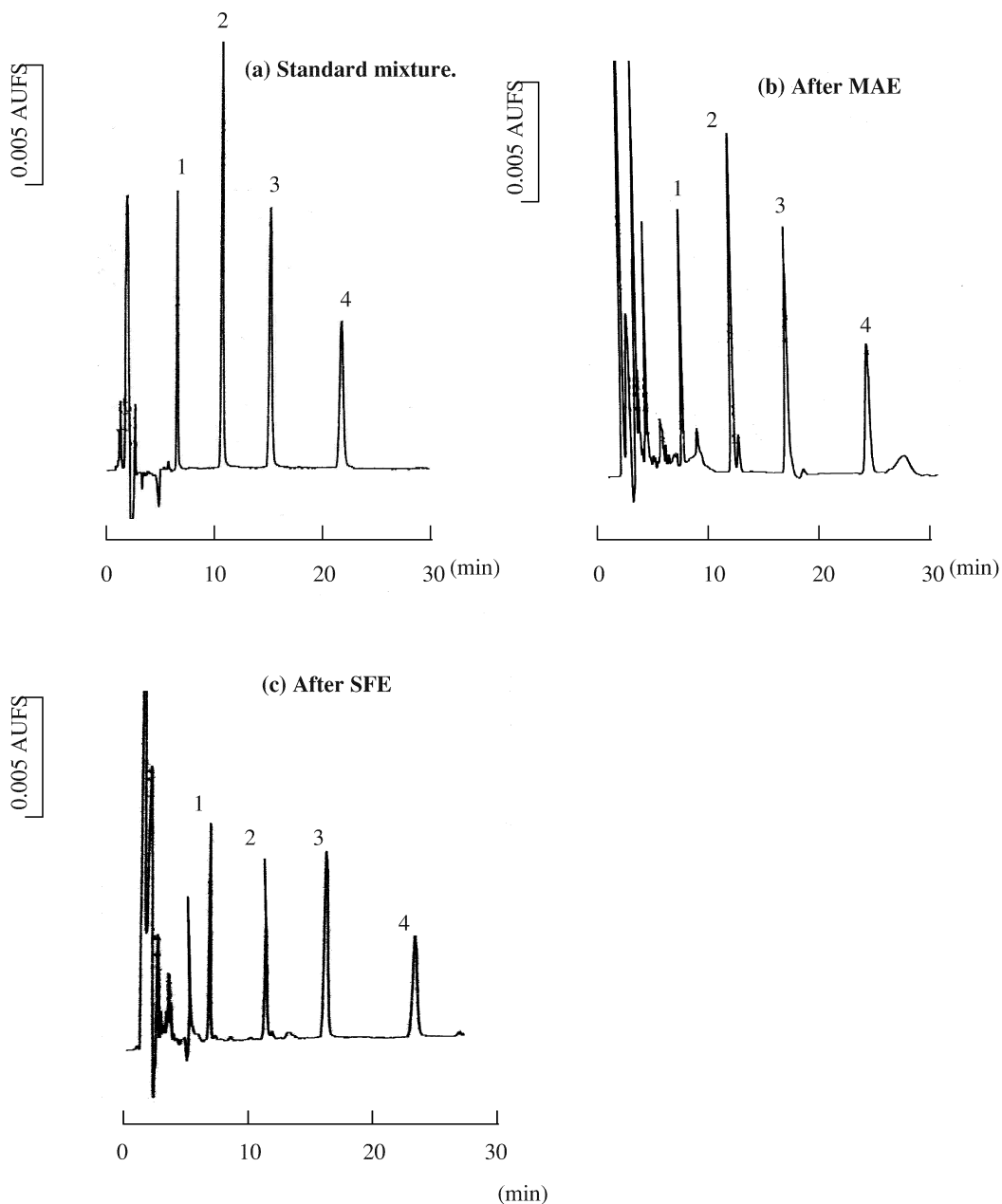


Fig. 2. (a) Chromatography of standard mixture of four carbamates; (b) Chromatography obtained after optimum MAE (6 min heating at 80 °C, methanol is the extraction solvent). Peak identities: (1) propoxur; (2) propham; (3) methiocarb; (4) chlorpropham; (c) Chromatography obtained after optimum SFE (300 kg/cm² of pressure, 60 °C of heating temperature, supercritical fluid: CO₂ with 10% methanol).

Table 8
Assignment table of variables and the arrangement of the experimental runs using an $OA_8 (2^7)$ matrix

Column no.						
1	2	3	4	5	6	7
A	B	^a	C	^a	B×C	D
0% Methanol	40 °C	30 min		200 kg/cm ²		
10% Methanol	60 °C	50 min		300 kg/cm ²		

A, Supercritical fluid; B, extraction temperature; C, extraction time; D, pressure.

^a Dummy factor.

(Fig. 2b). The figure demonstrated that excellent extraction efficiencies of tested carbamates were obtained after optimization of MAE. Thus, the employment of OAD for the optimization of procedural steps during MAE of soil samples has been shown to be advantageous. Moreover, it is both time- and cost-effective as trial-and-error steps can be greatly reduced after the optimum conditions are established.

3.4. Data analysis for SFE optimization

The conditions for the SFE technique should be optimized before carrying out any comparative study with other methods. On the basis of the previous work related to the SFE, the four variables selected for optimization of SFE conditions were: (1) supercritical fluid (factor A); (2) extraction temperature (factor B); (3) extraction time after equilibration (factor C); (4) pressure (factor D). The level setting

values of the main variables (A, B, C and D) used in two-level OAD are displayed in Table 8. According to previous experience and intuition, a two-variable interaction to be considered was B×C (interaction between different extraction temperatures and extraction time). Because four two-level variables and one two-variable interaction were to be considered, a total of five degrees of freedom is necessary and the $OA_8 (2^7)$ matrix was therefore chosen so as to have sufficient degrees of freedom for the assignment of the variables considered. The experimental design for optimization of SFE in this study is shown in Table 9. Furthermore, after implementing the eight experimental trials, the corresponding average recovery (AR) results for each experimental trial and the average responses for each factor at different levels were also calculated, as given in Table 9.

In principle, each column may be used to assign a factor. However, in order to measure the error variance, it is preferable for at least one column to be used to assign a dummy factor, in which no actual factor can be assigned. In this study, four actual variables and one-variable interactions are to be evaluated and assigned to columns 1, 2, 4, 6, 7. Thus, the $OA_8 (2^7)$ matrix would provide two dummy factors (columns 3, 5) that can be used for measuring the error variance. Consequently, the ANOVA table was constructed, as shown in Table 10. The table indicates that factor A (supercritical fluid), factor B (extraction temperature), and factor D (pressure) are statistically significant at equal to or above the 90% confidence level, whereas factor C

Table 9
The $OA_8 (2^7)$ matrix with the experimental results

Experiment no.	Column no.							Average recovery (%)
	1	2	3	4	5	6	7	
1	1	1	1	1	1	1	1	30.8
2	1	1	1	-1	-1	-1	-1	35.3
3	1	-1	-1	1	1	-1	-1	48.2
4	1	-1	-1	-1	-1	1	1	43.9
5	-1	1	-1	1	-1	1	-1	82.3
6	-1	1	-1	-1	1	-1	1	72.7
7	-1	-1	1	1	-1	-1	1	80.7
8	-1	-1	1	-1	1	1	-1	88.4
[I] ^a	39.6	55.3	58.8	60.5	60.0	61.4	57.0	
[III] ^a	81.0	65.3	61.8	60.1	60.6	59.2	63.6	

^a Average response of each level.

Table 10
Variance analysis table in the OA₈ (2⁷) matrix for the optimization of SFE

Parameter	A	B	C	D	B×C	Error
SS	3427.9	200	0.3	87.1	9.7	18.7
df	1	1	1	1	1	2
MS	3427.9	200	0.3	87.1	9.7	9.4
F-value	364.7	21.3	0.03	9.3	1.0	
P-value ^a	<0.05**	<0.05**		<0.1*		

SS, sum of squares; df, degrees of freedom.

^a The critical F-value is 18.51 (** $P < 0.05$) and 8.53 (* $P < 0.1$).

and the two-variable interaction B×C (interaction between extraction temperature and extraction time) have no significant influence on the optimization of SFE for the extraction of carbamate pesticides in soil. Minor differences are observed for the following variables: both factor A and B are significant at $P < 0.05$, while factor D is significant at $P < 0.1$.

Superiority and inferiority levels of the three significant factors were estimated by comparing the average response of factors at two levels. Table 9 gives the average response of these factors at levels 1 and 2, respectively. It is clear that the optimum levels are A (II), B (II) and D (II). From Tables 9 and 10, some very interesting conclusions are drawn: (1) The average recovery for extraction of carbamates from soil matrix with modified supercritical CO₂ has been greatly improved compared with neat CO₂. The average response of targets was 81.0% with the CO₂-10% methanol and only 39.6% with unmodified CO₂. This observation indicates that methanol increased the solvating power of non-polar CO₂ for the extraction of carbamates from spiked soil. From the ANOVA results given in Table 10, the fluid is identified to be the most significant of all the variables ($P < 0.05$); (2) Extraction temperature has been demonstrated to be another crucial factor for the recovery of polar compounds from soil samples: 55.3% of the average response of carbamate recovery was obtained at 40 °C; the recovery rose to 65.3% on raising the extraction temperature to 60 °C. The latter was thus selected as optimum. (3) The influence of pressure was significant for optimization of SFE conditions. Higher recovery was achieved under higher pressure of the SFE system; (4) Extraction time has little effect on SFE extraction in this study. Since target carbamates are thermally unstable, extraction time is set at 30 min to avoid

analyte degradation. Based on the above considerations, the optimum working conditions of SFE for the extraction of carbamates from spiked soil are 300 kg/cm² pressure and a temperature of 60 °C, and CO₂ modified with 10% methanol as the extraction fluid. After a few minutes equilibration, the 10-ml sample vial is extracted for 30 min. The HPLC chromatogram is presented in Fig. 2c, which shows analytes extracted from spiked soil under optimized SFE conditions. Fig. 2c shows that target carbamates are efficiently extracted from soil and good recoveries are obtained under the optimum conditions.

3.5. Comparison of MAE with SFE for extraction of tested carbamates

The optimized MAE and SFE techniques were employed separately for the extraction of carbamates in soil and the results compared. Fig. 3 shows the

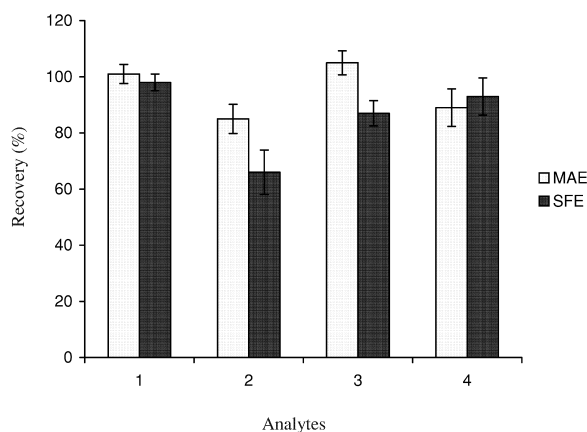


Fig. 3. Comparison of mean recoveries of four carbamate pesticides from spiked soil using MAE and SFE. Analytes: (1) propoxur; (2) propham; (3) methiocarb; (4) chlorpropham.

comparison of analyte recoveries obtained from both extraction methods. Tested carbamates in this figure, from left to right, are in the order of their chromatographic elution. From Fig. 3, it can be seen that in general, overall recovery of carbamates using MAE is higher than that using SFE. The recoveries by MAE and SFE ranged from 85 to 105% and from 66 to 98%, respectively. The overall average relative standard deviation (RSD) for all the targets was 5.2%. Fig. 3 also indicates that MAE is more suitable to extract methiocarb, for which 100% recovery under optimum MAE conditions is obtained, compared to 87.3% recovery by SFE. Both MAE and SFE achieve satisfactory extraction of propoxur since extremely high recoveries (101 and 98%, respectively) are obtained, SFE shows slightly higher recovery for chlorpropham (93 vs. 87% for MAE). For extraction of propham, MAE achieved ca. 85% recovery. What is surprising is that unusually poor recovery ($66.3 \pm 7.9\%$) for propham is obtained by SFE. This may be due to some degradation under the SFE conditions used.

To examine the effect of time-aged soil on the recoveries of carbamate pesticides by MAE and SFE,

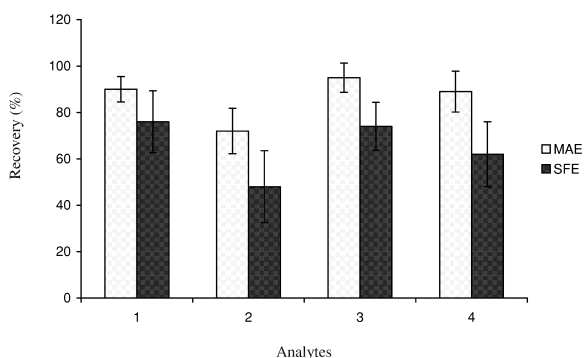


Fig. 5. Comparison of mean MAE and SFE recoveries of four carbamates from time-aged spiked soil.

spiked soil aged for 60 days was used for the investigations. Results are summarized and shown in Figs. 4 and 5. From Fig. 4, it can be noted that the extraction efficiencies of all the analytes by both methods decrease after aging. The overall average recovery by MAE dropped slightly from 95% (freshly spiked soil) to 87% (time-aged soil) and the overall average RSD was 7.6% compared to 4.9% (for freshly spiked soil). SFE data, however, showed a drop from 86 to 65% recovery when extraction was

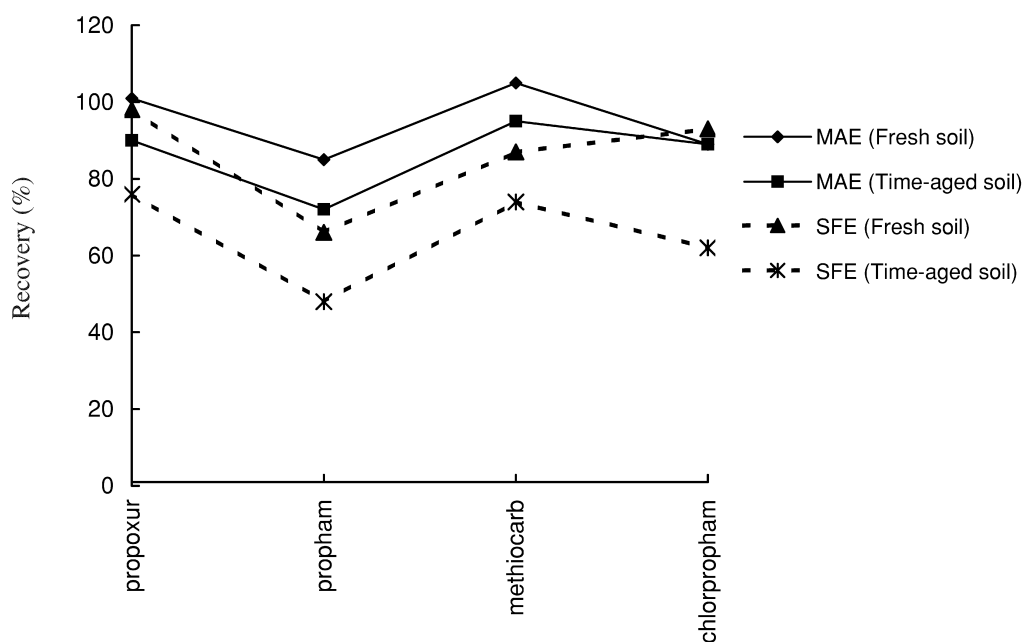


Fig. 4. Comparison of extraction efficiencies of carbamates from freshly spiked soil and time-aged soil by MAE and SFE. (1) Propoxur; (2) propham; (3) methiocarb; (4) chlorpropham.

from time-aged soil compared to fresh soil and the average RSD was 13.3 and 5.5%, respectively. Furthermore, it is also found from the figure that there is no marked effect of this parameter for chlorpropham extraction under MAE. According to Figs. 4 and 5, it is clear that MAE is an efficient technique for extracting carbamate pesticides from either freshly spiked or time-aged soil.

4. Conclusion

For the first time, an orthogonal array design was employed to optimize MAE and SFE conditions for extracting carbamates (propoxur, propham, methiocarb and chlorpropham) from spiked soil. An $OA_{16}(4^4)$ matrix derived from $OA_{16}(2^{15})$ was employed to optimize the MAE conditions. The average recovery of the carbamates was above 94% with good reproducibility under optimum MAE conditions. SFE was optimized by a two-level OAD procedure. Recoveries of the analytes from spiked soil by SFE ranged from 87 to 98%, except for propham (66%), which probably underwent thermal degradation under the extraction conditions. The results indicate that although carbamates tested in this study are thermally labile, there is little observable degradation under the optimum MAE and SFE conditions, except for propham. From the comparison of both methods, it is found that although higher recoveries were obtained by MAE, recoveries by SFE are still good (for propoxur, methiocarb and chlorpropham) under their respective optimum conditions. Therefore, both MAE and SFE are suitable techniques for extracting carbamate pesticides from spiked soil. However, when extraction is from time-aged soil, MAE exhibits better recoveries than SFE.

References

- [1] D.S. Saunders, C. Harper, in: A.W. Hayes (Ed.), Principles and Methods of Toxicology, Raven Press, New York, 1994, p. 389.
- [2] Y. Mason, E. Choshen, C. Rav-Acha, Water Res. 24 (1990) 11.
- [3] P.C. Gupta, J. Toxicol. Environ. Health 43 (1994) 383.
- [4] US Environmental Protection Agency, National Survey of Pesticides in Drinking Water Wells, Phase II Report, EPA 570/9-91-020, National Technical Information Service, Springfield, VA, 1992.
- [5] M. Fernández, Y. Picó, J. Mañes, J. Chromatogr. A 871 (2000) 43.
- [6] A. Dicorcia, C. Creszenci, A. Lagana, E. Sebastiani, J. Agric. Food Chem. 44 (1996) 1930.
- [7] D.A. Volmer, J. Chromatogr. A 794 (1998) 129.
- [8] S.B. Hawthorne, Anal. Chem. 62 (1990) 633A.
- [9] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. 66 (1994) 909.
- [10] M.L. Jeong, D.J. Chesney, Anal. Chim. Acta 389 (1999) 53.
- [11] I.A. Stuart, R.O. Ansell, J. Maclachlan, P.A. Bather, W.P. Gardiner, Analyst 122 (1997) 303.
- [12] K. Ganzler, A. Salgo, K. Valko, J. Chromatogr. 371 (1986) 299.
- [13] K. Ganzler, I. Szinai, A. Salgo, J. Chromatogr. 520 (1990) 257.
- [14] V. Lopez-Avila, R. Young, W.R. Bechet, Anal. Chem. 66 (1994) 1097.
- [15] I.J. Barnabas, J.R. Dean, I.A. Fowles, S.P. Owen, Analyst 120 (1995) 1897.
- [16] S.J. Stout, A.R. Dacunha, D.G. Allardice, Anal. Chem. 68 (1996) 653.
- [17] A. Pastor, E. Vazquez, R. Ciscar, M. de la Guardia, Anal. Chim. Acta 344 (1997) 241.
- [18] B. Lalere, J. Szpunar, H. Budzinski, P. Garrigurs, O.F.X. Donard, Analyst 120 (1995) 2665.
- [19] F.E. Onuska, K.A. Terry, Chromatographia 36 (1993) 191.
- [20] B.W. Renoe, Am. Lab. 8 (1994) 34.
- [21] K. Ganzler, A. Salgo, Z. Lebensm. Unters. F.A. 184 (1987) 274.
- [22] E. Hasty, R. Revesz, Am. Lab. 2 (1995) 66.
- [23] V. Lopez-Avila, J. Benedicto, C. Charan, R. Young, W.F. Bechet, Environ. Sci. Technol. 29 (1995) 2709.
- [24] V. Lopez-Avila, R. Young, J. Benedicto, P. Ho, R. Kim, W.F. Bechet, Anal. Chem. 67 (1995) 2096.
- [25] P.J. Oles, A. Yankovich, LC-GC 7 (1989) 579.
- [26] W. Chariteris, J. Soc. Dairy Technol. 45 (1992) 33.
- [27] P.J. Oles, G. Gates, S. Kensinger, J. Patchell, D. Schumacher, T. Showers, A. Silcox, J. Assoc. Off. Anal. Chem. 73 (1990) 724.
- [28] P.J. Oles, J. Assoc. Off. Anal. Chem. 76 (1993) 615.
- [29] P. Bilot, B. Pitard, J. Chromatogr. 623 (1992) 305.
- [30] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok, Y.H. Poh, J. Chromatogr. A 677 (1994) 255.
- [31] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1659.
- [32] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1669.
- [33] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok, Anal. Chim. Acta 289 (1994) 371.
- [34] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Talanta 41 (1994) 1917.
- [35] W.G. Lan, K.K. Chee, M.K. Wong, Y.M. Sin, Analyst 120 (1995) 273.

- [36] W.G. Lan, K.K. Chee, M.K. Wong, H.K. Lee, Y.M. Sin, *Analyst* 120 (1995) 281.
- [37] K.K. Chee, M.K. Wong, H.K. Lee, *J. Chromatogr. A* 723 (1996) 259.
- [38] Y. He, H.K. Lee, *J. Chromatogr. A* 793 (1998) 331.
- [39] L. Sun, H.K. Lee, *J. Sep. Sci.* 25 (2002) 1.
- [40] D.C. Montgomery, *Design and Analysis of Experiments*, Wiley, New York, 1997.