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Combination of microwave assisted micellar extraction and liquid chromatography for the determination of organophosphorous pesticides in soil samples

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Abstract

A new methodology based on the microwave assisted micellar extraction (MAME) technique has been optimised, using soil samples, to extract and determine a mixture of the eight organophosphorous pesticides mainly used in agriculture. The pesticides under study have been extracted using the non-ionic surfactants polyoxyethylene 10 lauryl ether (POLE) and oligoethylene glycol monoalkyl ether (Genapol X-080). The optimal extraction variables, such as surfactant concentration, pH, radiation time and microwave power were determined for each surfactant. The results show the advantage of using POLE instead of Genapol X-080 for the extraction of the organophosphorus pesticides with recoveries higher than 70% for most of the compounds and relative standard deviations (RSD) below 2.6%. This method was successfully applied to fresh samples as well as to aged samples for the analysis of soils with different characteristics and compared with the traditional Soxhlet technique.

Keywords: Organophosphorous pesticides; Surfactants; Microwave assisted micellar extraction; HPLC–UV

1. Introduction

Organophosphorus pesticides are effective against a great variety of insects. They are used in agricultural crops, residential and commercial buildings, ornamental gardens and plants and also to control the presence of disease-carrying mosquitoes. [1]

Their wide use could lead to extensive pollution of the environment and constitutes a potential and/or deliberate risk to human health [2–5]. Amongst the acute effects caused by intentional or accidental overdoses and high doses of exposure to organophosphorous compounds is neurological dysfunction [6].

Among the different procedures employed in the extraction of organic pollutants from solid samples, the traditional Soxhlet extraction has undoubtedly been the most widely used [7–9]. However, this method has a series of drawbacks, as it is time consuming (between 24 and 48 h) and needs large amounts of organic solvents (100–300 ml) that have to be evaporated before further clean-up steps.

In the last decade, there has been an increasing demand for new extraction techniques, amenable to automation, with shortened extraction times and reduced organic solvent consumption – preventing pollution in analytical laboratories – and reducing sample preparation costs [10]. Driven by these objectives advances in sample preparation have resulted in a number of techniques such as supercritical fluid extraction (SFE) [11–13], pressurised liquid extraction (PLE) [14] and microwave-assisted extraction (MAE) [15–17].

Domestic microwave ovens were used by Ganzler et al. as early as 1986, [18] to extract anti-nutritive compounds from various plant materials. Since then, microwave methodologies have been adapted for other scientific applications,

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including the extraction of pesticides [19,20], metals [21], PAHs [22], and many other pollutants [23]. Although the advantages of these procedures include reduced solvent usage and shorter analysis time, most of them still make use of organic solvents. Thus, to completely avoid the use of these extractants, there is the possibility of a new application of microwave-assisted extraction using biodegradable micellar media as extractants.

From an analytical point of view, one of the most important properties of these organised structures is their good capacity to solubilise solutes of different types found in different environments [24,25]. Therefore, the combination of the MAE technique with the use of micellar media make this a simple, fast, low cost, easy handling and non-toxic procedure (MAME), which could be an alternative for the extraction of different pollutants from solid matrices as has recently been proven [26–28].This paper describes the performance of MAME methodology in the extraction of eight organophosphorous pesticides using two different surfactants, polyoxyethylene 10 lauryl ether (POLE) and oligoethylene glycol monoalkykl ether (Genapol X-080), and their following determination by liquid chromatography with UV detection. The performance and application of this method on soils is important because of the difficulty in extracting the organophosphorous pesticides from such complex matrices. In fact there are very few publications that cover these types of matrices. The proposed methodology offers a method that is quick, simple and free of organic solvents.The optimised methodology was successfully applied to the analysis of soils in fresh samples as well as in aged samples, with different characteristics. The compounds can be extracted more selectively and more quickly with similar or better recoveries in comparison with conventional extraction processes.

2. Experimental

2.1. Reagents

Pesticide standards (dimethoate, methidathion, parathion methyl, malathion, ethoprophos, parathion ethyl, diazinon, chlorpyrifos) were provided by Dr. Ehrenstorfer (Augsburg, Germany). All pesticide standards were of 98–99% purity and are listed in Table 1, which sets out their identification numbers in tables and figures. Stock solutions of each pesticide were prepared in methanol at 100 μg mL^{-1}.

Table 1
Analytes under study

Compound	Identification number	t_r[a] (min)	λ (nm)
Dimethoate	1	4.5	205
Methidathion	2	9.6	215
Parathion-methyl	3	10.2	271
Malathion	4	10.7	215
Ethoprophos	5	11.9	228
Parathion-ethyl	6	13.2	276
Diazinon	7	14.3	247
Chlorpyrifos	8	21.2	228

[a] Retention time.

The non-ionic surfactants polyoxyethylene 10 lauryl ether and oligoethylene glycol monoalkyl ether (Genapol X-080) were obtained from Sigma (St. Louis, MO, USA) and prepared in ultra-high quality water.

HPLC-grade methanol was obtained from Panreac Quimica, S.A. (Barcelona, Spain) and was used as received.

All solvents were filtered through a 0.22 μm nylon membrane filter, and ultra-high quality water was used throughout.

2.2. Apparatus

The microwave system used to perform the microwave-assisted extraction (MAE) process was a Multiwave (Perkin-Elmer, Madrid, Spain), with a rotor 6EVAP and 6 MF100 vessels (Perkin-Elmer, Madrid, Spain).

A pH-meter (Crison, Spain) was used for the characterization of the soil samples.

The HPLC system was equipped with Millenium chromatography manager software, a Waters 515 pump (Waters Associates, Milford, MA), fitted with a Rheodyne 7725i injector valve, and a Waters 996 photodiode array detector (Waters Associates).

The column was a Waters Nova-Pack C_{18} 150 mm × 3.9 mm, 4 μm particle diameter (Waters Associates).

2.3. Procedure

2.3.1. Characterization of the samples: organic matter and pH determination

The Sauerlandt method [29] was used to determine the organic matter content in the samples, which comprises the oxidation of the organic matter using potassium dichromate and sulphuric acid.

The Official Method 994.18 of the AOAC [30] was followed to determine the pH by measuring it in a suspension created by agitating the sample in water.

2.3.2. Spiking of samples

The kind of soil that was employed for the optimization of the extraction procedure had the following characteristics: pH 8.3, organic matter content 3.4% and granulometric distribution—250 μm: 20.1%; 125 μm: 16.9%; 0.0625 μm: 6.9%; <0.0625 μm: 5.8%.

Two grams of soil sample were spiked with the pesticide mixture, shaken and stored overnight in the dark in order to obtain a dry and homogeneous sample.

2.3.3. Microwave assisted micellar extraction

Spiked samples were introduced into Teflon vessels adding different solutions containing the non-ionic surfactants at the optimised concentrations. The vessels were placed in the microwave oven, irradiated at the optimised conditions and then allowed to cool to room temperature. The surfactant extracts were carefully removed, filtrated and introduced

into hermetically closed vials before their analysis in the HPLC–UV system.

2.3.4. Soxhlet extraction

Two grams of the spiked samples were extracted with hexane:acetone (1:1) for 24 h at 4–6 cycles/h as proposed by the EPA Method 3540 C [31]. The extract was evaporated in vacuo, redissolved in methanol (10 mL) and finally analysed in the HPLC–UV system.

2.3.5. Chromatographic analysis

Twenty microlitres of the extracted solutions were analysed in the LC–UV system, with different wavelengths being recorded in each case (Table 1). The following were the optimised conditions used for the separation and identification of all the analytes under study. A mobile phase of methanol:water (35:65) during 2 min, then gradient until 6 min to methanol:water (70:30), isocratic until 13 min and then gradient until 25 min to methanol:water (99:1). A constant flow rate of 1 mL min^{-1} was maintained during all the analysis.

The conditions employed were the same for the analysis of both the MAME and Soxhlet extracts.

3. Results and discussion

3.1. Optimization of the microwave assisted micellar extraction

3.1.1. Effect of extractant volume

A preliminary study was made in order to check if the volume of extractant to be added would effect the extraction of the analytes due to possible evaporation losses or a non-complete interaction with the sample. In this way, measurements of the analyte recoveries were performed using 5, 10 and 20 mL of Genapol X-080 solution (4%, v/v) as well as POLE at a concentration of 5% (v/v). At extraction volumes lower than 5 mL irreproducible data were obtained (due to the insufficient covering of the extractant) and at volumes higher than 20 mL some evaporation losses took place (due to the high temperatures reached). This last effect maybe due to the high capacity of the aqueous surfactant solutions in absorbing the microwave radiation and transforming it into heat [32]. In the range of volume studied no significant differences were observed for any of the two surfactants.

Thus, a volume of 10 mL was chosen for following studies in order to ensure the sample was totally covered by the surfactants.

3.1.2. Effect of surfactant concentration

In order to determine the effect of surfactant concentration on the recovery percentage, several samples containing different POLE and Genapol X-080 concentrations, 1, 3, 5 and 7% (w/v), were analyzed. The pesticides from enriched samples were extracted with both surfactant solutions in the microwave system at 300 W during 5 min.

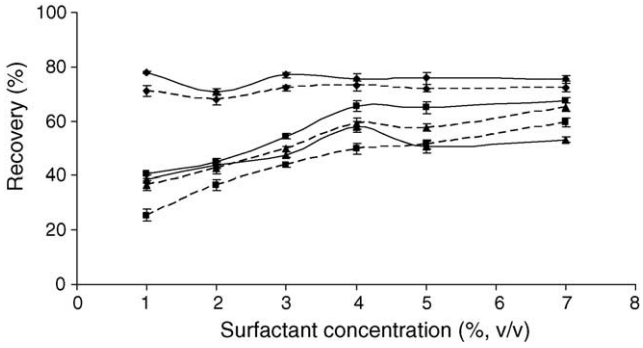

Fig. 1. Effect of surfactant concentration on the recovery of the pesticides under study. Continous line (—): Genapol X-080; dashed line (- - -): POLE. (1) Dimethoate, (3) parathion-methyl and (7) diazinon.

The recoveries obtained for some of the compounds studied can be seen in Fig. 1. It can be seen how the two surfactants studied behave in the same way. For those more polar analytes such as Dimethoate, the recuperation is practically constant in the range of concentrations studied, while the recoveries of less polar compounds rises until a concentration of 4% (v/v) after which they are maintained practically constant. For later studies concentrations of 4% for Genapol X-080 and 5% (v/v) for the POLE were used.

3.1.3. Effect of the pH

The effect of the pH on the pesticide recoveries was determined by analysing the extracts of spiked samples when using POLE or Genapol X-080 solutions as extractants at different pH. In each case it was changed by adding 0.5 mL of HCl (1 M) or NaOH (0.1 M) solutions. The soil samples were irradiated in the microwave oven at 300 W during 5 min and analysed, after their filtration, in the HPLC system.

In a qualitative way, it was observed that the extracts obtained were darker when using NaOH than those using HCl, and in fact they were even darker than those extracted with only surfactant. The pH of these extracts was similar for both surfactants, for the acid extracts 3.3–3.8 and for the basics of 8.2–8.6. Coinciding with the colour of the extracts and with the difference of pH it can be seen that the recoveries were higher in the majority of cases when NaOH was added (Fig. 2) and lower when HCl was added independent to the sufactant used. Only in the case of Dimethoate no significant differences were observed in the recuperations obtained by the different extractants, demonstrating that it is not affected by changes in the pH of the solution.

Having observed these results, it was decided to add 0.5 mL of NaOH (0.1 M) in later studies.

3.1.4. Effect of the microwave radiation power and time

As the temperature obtained inside the vessels is the parameter that determines the efficiency of extraction, and this

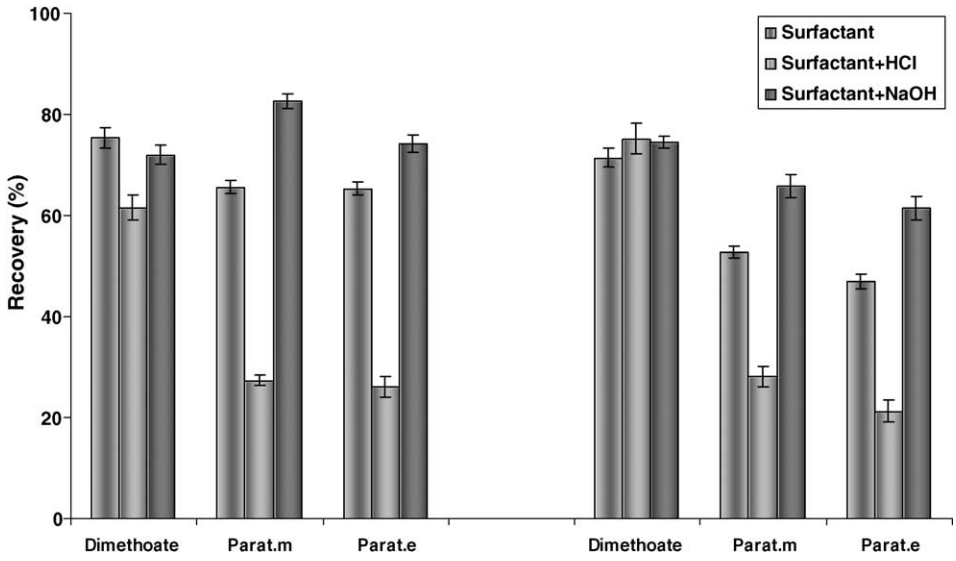

Fig. 2. Effect of pH on the recovery of the analytes under study extracted with both surfactants: (a) Genapol X-080 and (b) POLE.

depends on the radiation time and power applied, both variables were analyzed at the same time.

A central composite design was followed in order to study the effect on the recovery. A two-level full factorial design, 2^2, with a star orthogonal composite design and three central points (11 runs in total) allowed the direct evaluation of the considered variables [33]. Therefore, a group of soil samples were analyzed using the previously optimised conditions for each surfactant, at different microwave powers, ranging from 200 to 800 W, and radiation times from 2 to 14 min. Table 2 shows the different radiation conditions used for each run. For diazinon in Genapol X-080 (Fig. 3a) the results obtained show a maximum for intermediate powers and short times. The behaviour was similar for the rest of the pesticides that were extracted with this surfactant. In the case of POLE the better recoveries are obtained at higher powers and in a short time. This can be seen in Fig. 3b, that shows the surface diagram for parathion-methyl. But in order to determine more precisely the optimum time and microwave power, the equation that was closest to the behaviour of each pesticide in each surfactant was used to calculate the percentage of maximum recuperation for each analyte in both surfactants. Indeed the averages obtained demonstrated radiation times of 2 min in both surfactants and radiation powers of 450 and 625 W for Genapol X-080 and POLE, respectively.

Finally, the extraction efficiency under optimum conditions was tested for each surfactant demonstrating that in general POLE was better for extracting pesticides than Genapol X-080.

Table 2
Runs employed for the study of the effect of the radiation conditions over the recovery

Run number	Power (W)	Time (min)
1	100	2
2	100	8
3	100	14
4	450	2
5	450	8
6	450	8
7	450	8
8	450	14
9	800	2
10	800	8
11	800	14

3.2. Analytical parameters

The corresponding calibration curves were obtained by injecting standard solutions containing a known concentration of the pesticide mixture and the surfactant POLE or Genapol X-080 into the chromatographic system. The results revealed in the case of both surfactants a linear relationship in the interval 100–2500 ng mL^{-1} with high correlation coefficients (0.999) for all pesticides.

In order to study the repeatability, the optimised method was applied to the analysis of six samples containing the mixture of pesticides which were determined at the established chromatographic conditions. The relative standard deviation (RSD) values are listed in Table 3, where values equal and lower than 2.1 and 2.6% were obtained for Genapol X-080 and POLE respectively. Fig. 4 shows the chromatogram of the pesticide mixture extracted from a soil sample under the optimised conditions for POLE.

The limits of detection were also calculated, once the MAME method was fully applied, for each analyte using the signal to noise (s/n = 3) ratio [34]. The results obtained are also listed in Table 3.

In order to probe the validity of the optimised MAME method, and due to the lack of a certified material available

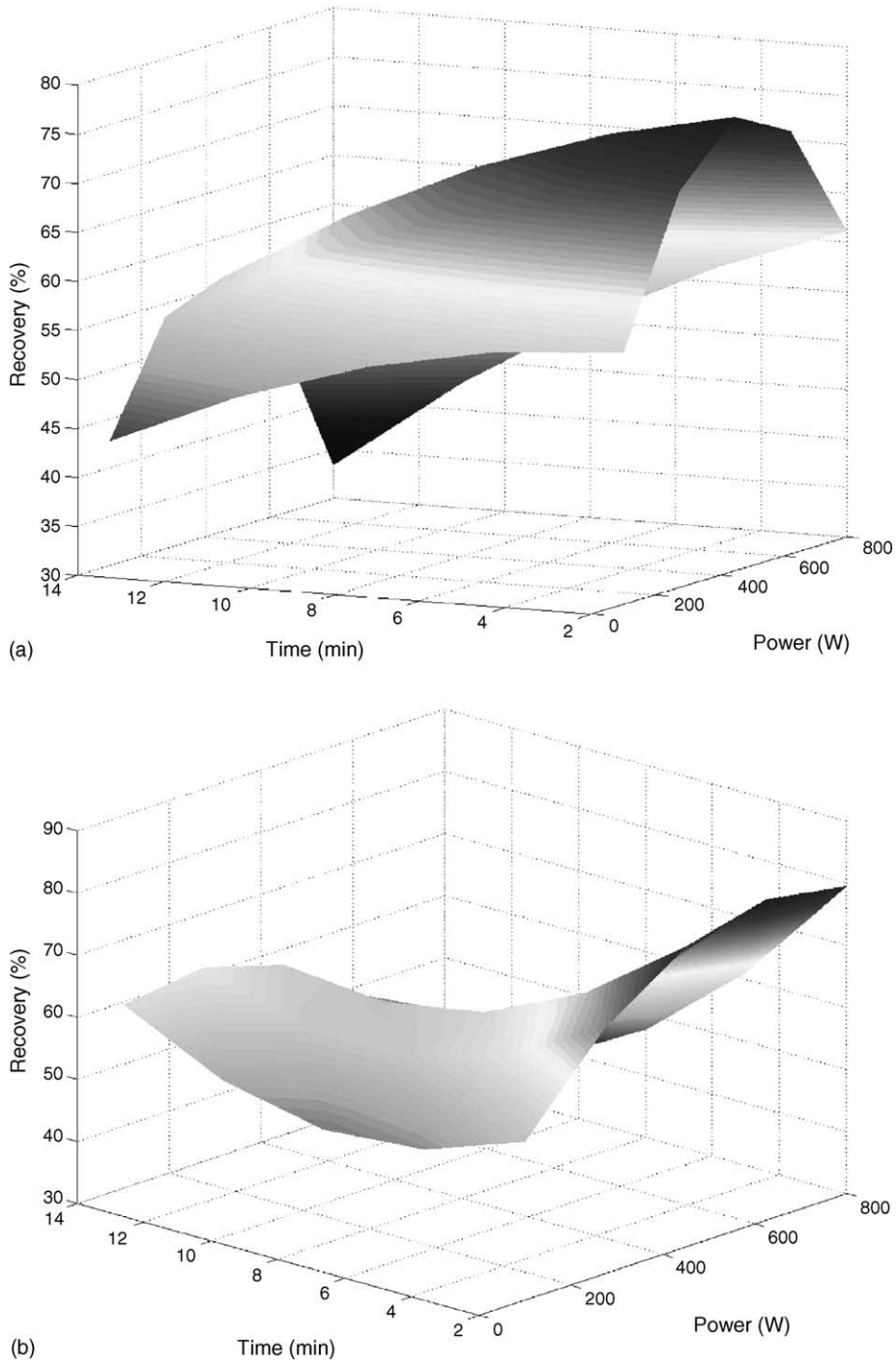

Fig. 3. Effect of microwave radiation time and power on the recovery of: (a) diazinone using Genapol X-080 as extractant and (b) parathion-methyl using POLE as extractant.

containing these pesticides, it was applied to the extraction of the pesticides present in a soil sample enriched with a certified mixture (Pesticide Mix 1, EPA Method 914), obtaining the results shown in Table 4.

These results were compared with those obtained using the traditional Soxhlet extraction procedure as proposed by the EPA in the 3540 Method [32], finding an important similarity of results in the two methods.

3.3. Analytical applications

The method using both surfactants was applied to several natural soil samples collected from Gran Canaria island (Canary Islands, Spain), with different values of acidity, organic content, and granulometry as can be observed in Table 5.

In the first instance, blanks from the different samples were analysed to ensure the absence of the compounds to be stud-

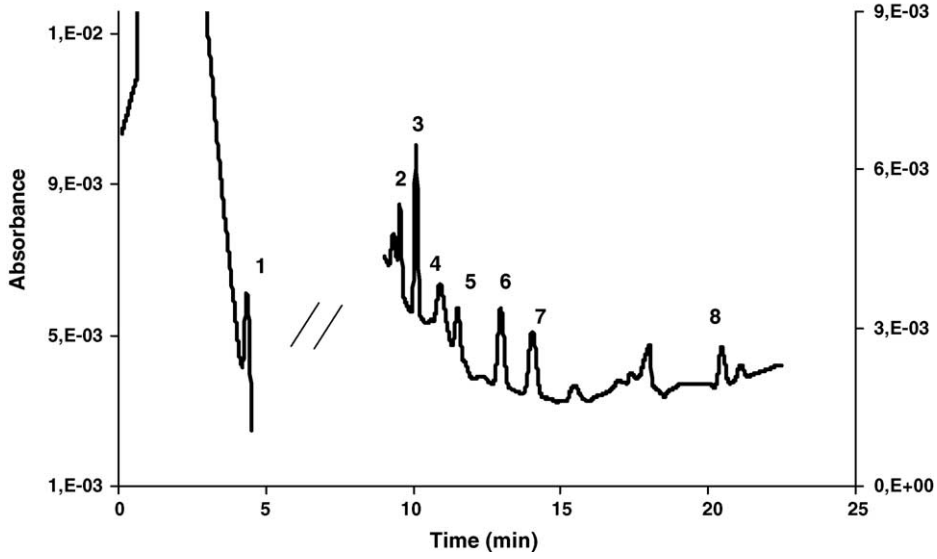

Fig. 4. Chromatogram of the organophosphorous pesticide mixture extracted from soil sample n° 1 with POLE at the optimized conditions. Chromatographic conditions specified on the text.

Table 3
Analytical parameters

Compound	POLE		Genapol X-080	
	RSD[a] (%)	LOD[b] (ng mL^{-1})	RSD[a] (%)	LOD[b] (ng mL^{-1})
Dimethoate	0.4	0.2	1.8	2.4
Methidathion	2.2	4.5	2.1	3.0
Parathion-methyl	1.1	2.1	1.2	4.8
Malathion	2.2	14.3	1.1	11.3
Ethoprophos	2.6	95.0	1.0	0.8
Parathion-ethyl	1.6	0.7	1.6	1.8
Diazinon	1.2	2.5	0.8	0.9
Chlorpyrifos	0.3	6.7	1.1	0.2

[a] Relative standard deviation ($n = 6$).
[b] Limit of detection.

Table 4
Application of MAME procedure and Soxhlet extraction to a soil sample containing a certified mixture of pesticides, (Pesticide Mix 1, EPA Method 914)[a]

Compound	MAME		Soxhlet	
	Added (μg g^{-1})	Found (μg g^{-1})	Added (μg g^{-1})	Found (μg g^{-1})
Parathion-methyl	1.50	1.39 ± 0.02	2.00	2.04 ± 0.02
Malathion	1.50	1.27 ± 0.04	2.00	1.89 ± 0.11
Parathion-ethyl	1.50	1.49 ± 0.12	2.00	1.85 ± 0.02
Diazinon	1.50	1.46 ± 0.02	2.00	1.64 ± 0.05

[a] Mean of three determinations.

ied. Later, the most adequate conditions for each surfactant were applied to samples enriched with a mixture of pesticides with concentrations between 500 and 2000 ng g^{-1}. The results obtained from the different samples can be observed in Table 6.

In general the extraction efficiency is better when POLE is used on samples n° 1 and n° 3 that present a basic pH, although the organic matter content and granulometry are different.

When the samples have the same pH but different organic matter content, the recoveries decrease with increasing organic matter content for both of the surfactants as it can be appreciated when comparing samples n° 1 and n° 3 or n° 2 with n° 4. Moreover, the texture of a soil is extremely important in the sorption process. When the particles are small they present a high superficial area thus increasing the adsorption,

Table 5
Physico-chemical characteristics of the different soil samples

Samples	pH	O.M. (%)[a]	Granulometry (%)			
			250 μm	125 μm	0.0625 μm	<0.0625 μm
Soil n° 1	8.3	3.4	69.9	16.9	6.9	5.8
Soil n° 2	5.9	3.9	56.5	30.6	6.8	6.0
Soil n° 3	8.3	12.5	45.8	32.1	12.0	10.1
Soil n° 4	5.4	6.2	33.0	34.0	16.1	16.9
Soil n° 5	4.8	4.4	40.0	31.7	13.5	14.8
Soil n° 6	3.9	6.2	25.4	26.6	23.6	24.5

[a] O.M.: organic matter content.

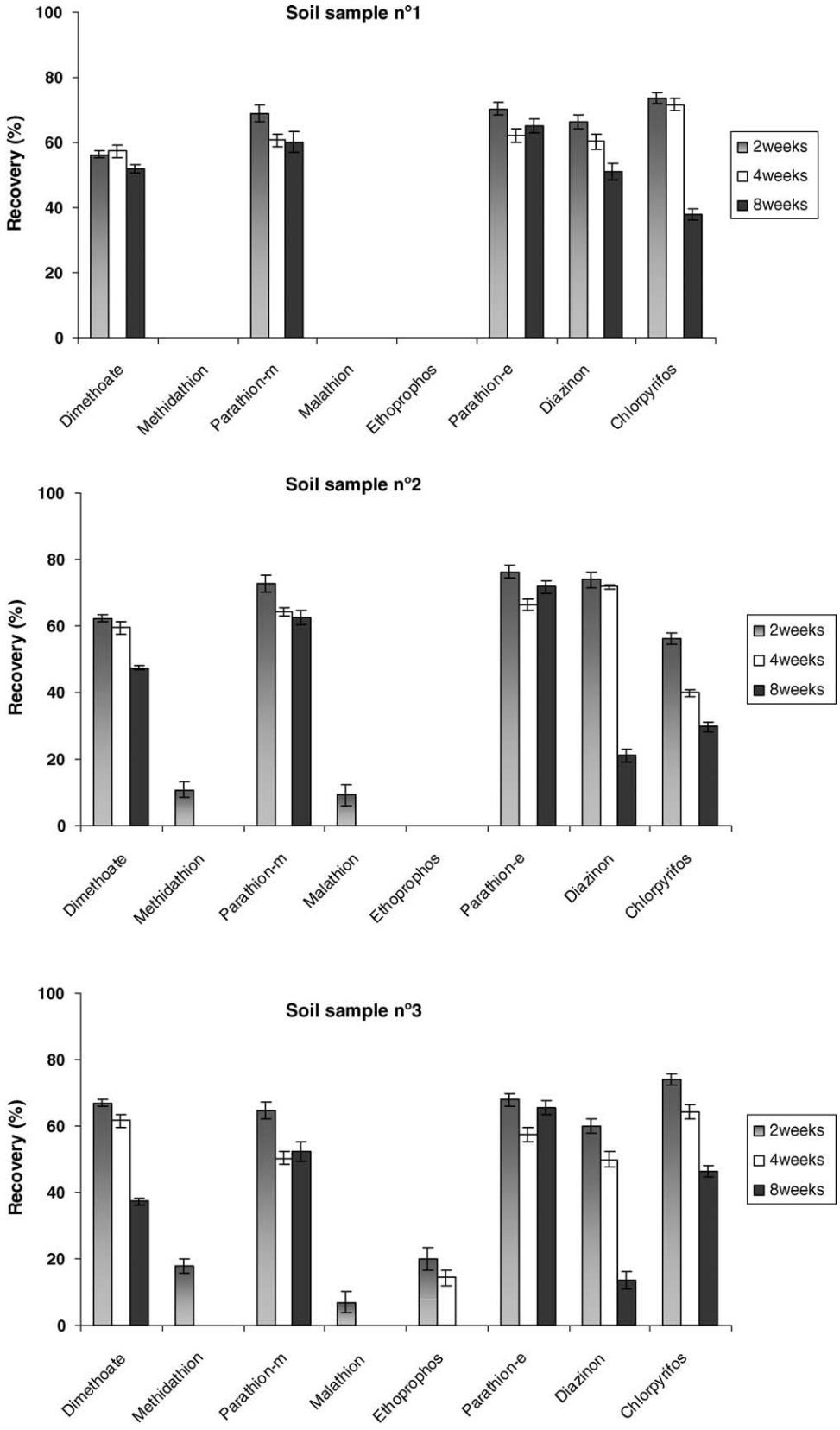

Fig. 5. Recoveries of pesticides from aged samples after MAME procedure with POLE.

Table 6
Application of the optimized MAME methodology to soil samples with different physico-chemical characteristics

Surfactant	Compound	Recovery (%)[a]					
		Soil n° 1	Soil n° 2	Soil n° 3	Soil n° 4	Soil n° 5	Soil n° 6
Genapol X-080	Dimethoate	82.3	67.3	66.1	36.9	58.64	57.85
	Methidathion	52.1	41.1	46.1	85.07	52.47	52.11
	Parathion-methyl	83.5	68.5	73.3	31.23	48.47	49.06
	Malathion	61.9	58.6	78.3	53.38	41.72	72.55
	Ethoprophos	89.6	83.9	24.2	50	96.77	87.78
	Parathion-ethyl	68.5	54.3	62.4	34.89	46.92	60.41
	Diazinon	20.5	–[b]	10.8	44.69	49.38	52.5
	Chlorpyrifos	74.8	66.1	75.1	53.94	49.4	49.09
POLE	Dimethoate	73.4	63.6	79.2	45.95	–[b]	40.87
	Methidathion	79.2	25.0	94.8	77.77	83.3	59.65
	Parathion-methyl	84.3	60.7	51.2	25.91	50.62	52.32
	Malathion	57.2	–[b]	40.1	58.4	61.18	60.62
	Ethoprophos	74.4	91.2	82.1	105.9	22.18	32.56
	Parathion-ethyl	87.6	89.0	56.5	23.13	36.33	54.73
	Diazinon	66.6	59.7	48.9	30.56	37.38	59.48
	Chlorpyrifos	85.1	86.5	85.6	81.43	73.73	80.42

[a] Mean of three determinations.
[b] (–) Non-extracted compounds.

although this adsorption process depends on the nature of the analytes. In this sense, a high variability can be observed in the recoveries obtained with the different granulometry of the soil samples. In general, it can be said that the proposed method is applicable in the sense that it enables the extraction of the pesticides under study even if the recovery efficiency depends on the characteristics of the soil as well as the surfactant to be used.

The effect of ageing the samples on the recovery of the analytes is a known phenomenon [35]. In order to test this effect, the optimised MAME procedure using POLE as an extractant was applied to soil samples aged to different times, 2, 4 and 8 weeks. The results obtained for soil samples n° 1, 2 and 3 can be seen in Fig. 5. Using different ageing times it can be observed that as the contact time increases between the analyte and the matrix the general recuperation of all the analytes under study was reduced which could be explained by the greater interaction between them [36]. This effect could also be caused by a process of degradation because as is well known the degradability of this family of compounds is far greater than that of organoclorine pesticides. Among the different pathways of organophosphates decomposition (hydrolysis, photolytic oxidation, microbial transformations, etc.) [37,38], hydrolysis is the most common degradation process which can occur at several reactive centres in a given organophosphorus pesticide molecule [39]. In this sense it can be observed that the compounds methidathion, malathion and ethoprophos are not detected after 2 weeks. This could be explained by the different degradation mechanisms including the presence of humic acids, metal oxides and the soil structure [38–41]. In addition the disappearance of these compounds is even more evident in soil n° 2 that has the lowest pH which could confirm the hydrolysis process.

However, this tendency is not the same for all the compounds being studied. The recuperation of parathion-methyl and parathion-ethyl remains approximately constant after 4 weeks and this could be interpreted as showing these compounds to be the more persistent. On the other hand, and from a comparative point of view, no significant differences can be appreciated in the recuperation of analytes extracted from the different soils but that in fact the effect of ageing, effects the recuperation of all the analytes over time equally. Therefore, we can conclude that the method is applicable to aged samples in the sense that is possible to determine the presence of the pesticides although the recoveries decrease.

4. Conclusions

This study proves the suitability of the non-ionic surfactants, in this case as extractants of compounds with different polarities like the pesticides under study. In our case, the results obtained for both surfactants, POLE and Genapol X-080, are in general satisfactory, overall in the case of freshly spiked samples. Nevertheless, it is important to emphasize the strong dependence of the recoveries on the physico-chemical characteristics of the soil samples.

The combination of the extraction procedure using surfactants as extractants with the microwave assisted extraction makes the method more rapid and less extractant is needed, thus lowering the costs dramatically. It is relatively simple, in so far as it does not require a high level of handling and the extract can be analysed directly. Moreover, it can be applied to the extraction of several samples at the same time and it has no toxical effects.

It can therefore be considered that this method is promising and may be a good alternative to the traditional techniques

usually employed to extract compounds in this kind of samples.

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