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Article

Solid Phase Microextraction as an Efficient Method for Characterization of the Interaction of Pesticides with Different Soil Types

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Foi desenvolvido um método de microextração em fase sólida (SPME) para determinação simultânea de 20 pesticidas, pertencentes a vários grupos, em amostras de solo. Foram investigadas condições de extração como tipo de fibra, temperatura e tempo de dessorção, tempo de extração e teor de NaCl. A detecção e quantificação foram realizadas por cromatografia gasosa acoplada a espectrometria de massas (CG-MS). Os resultados indicam que a maioria dos pesticidas estudados foram mais fortemente sorvidos por solos com maior teor de matéria orgânica ou argila. Os valores de desvio padrão relativo (DPR) para análises múltiplas de amostras de solo fortificadas com 30 μg kg^{-1} de cada pesticida ficaram abaixo de 19%. Os limites de detecção (LD) para todos os compostos estudados foram menores do que 5 μg kg^{-1}.

A solid phase microextraction (SPME) method for simultaneous determination of 20 pesticides belonging to various pesticide groups in soil samples was developed. Extraction conditions, such as fibre type, desorption temperature and time, extraction time and NaCl content were investigated. Detection and quantification were done by gas chromatography-mass spectrometry (GC-MS). The results indicate that most of the studied pesticides were more strongly sorbed by soil that has higher organic matter and/or clay content. Relative standard deviation (RSD) values for multiple analysis of soil samples fortified at 30 μg kg^{-1} of each pesticide were below 19%. Limits of detection (LOD) for all the compounds studied were less than 5 μg kg^{-1}.

Keywords: solid phase microextraction, pesticides, soil, multiresidue method

Introduction

Soil contamination is a global environmental pollution problem. One of the major soil contaminants are pesticides and their degradation products, which could cause serious problems for crops, soil organisms and humans.

Extraction is the most time-consuming and difficult segment of chemical analysis of these compounds in complex sample matrices like soil. Generally, routine procedures such as liquid-liquid extraction (LLE), soxhlet extraction and solid phase extraction (SPE) are time-consuming, tedious, require large quantities of organic solvents and are often relatively expensive. Therefore, recent trends in sample preparation have focused on a development of simpler, faster, more reliable and cost-efficient methods by reducing analysis time and solvent consumption. Solid phase microextraction (SPME), as a technique which combines extraction and concentration processes into one step, is the example of such development.

SPME is a simple, selective and efficient sorption/desorption method, based on the analytes' distibution between the sample matrix and extraction medium. Extraction is performed in a thin polymer film coating of a fused silica fibre, which is either immersed in a sample (DM-SPME) or exposed to a headspace above the sample (HS-SPME). After extraction, the fibre carrying sorbed analytes is introduced into a gas chromatograph injector for thermal desorption (GC), while in the case of liquid chromatography (LC) the analytes are desorbed by solvent elution.

So far, there have been scarce references to SPME application to determine pesticides in soil. Most of them are based on preparation of soil mixtures with distilled water and subsequent immersion of the SPME fibre in this

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slurry[1] or its exposing to a gas phase above the slurry.[2-7] Some researchers have suggested that DM-SPME of a soil organic extract obtained by solid-liquid extraction diluted with appropriate amount of water is the most reliable soil SPME method.[8-10] Their results indicate that this approach is more sensitive and provides both higher recoveries and better linearity. Most of these proposed methods, however, focus on simultaneous determination of pesticides belonging to only one or two pesticide groups. To our knowledge, there is actually only one report on SPME determination of pesticides that belong to several pesticide groups (chloroacetanilide, pyrethroid, organochlorine and organophosphorus compounds).[7] This method is based on headspace analysis of soil samples wetted with ultrapure water (50%, m/v).

As no previous studies are known to us dealing with the DM-SPME determination of pesticides of different pesticide groups, the aim of this study was to develop a rapid and simple DM-SPME multiresidue method for simultaneous determination of 20 compounds having distinct chemical structures and belonging to different pesticide groups. The main parameters affecting DM-SPME procedure, such as the fibre type, temperature and time of desorption, extraction time and NaCl content, as well as the extraction efficiencies of several solvents (water, hexane, acetonitrile, acetone and methanol) and the optimum number of extraction steps within the sample preparation step were investigated and optimized. Additionally, the proposed method was used to determinate the several selected pesticides in different soil types in order to examine whether, why and to what extent different soil properties are able to effect SPME efficiency.

Experimental

Reagents and materials

Pesticides chosen for this study were: clomazone, acetochlor, pendimethalin, oxyfluorfen, prometryn, fenitrothion, chlorpyrifos, lindane, (Dr Ehrenstorfer); dimethenamid, chloridazon (BASF); phorate (City Chemical Corporation); simazine (Ciba-Geigy); atrazine (Syngenta); fluorochloridone (Chimac Agriphar S.A.); tebupirimfos, metribuzin, tebuconazole (Bayer); terbufos (Counter); chlorothalonil (Pestanal) and prochloraz (Aventis) (Table 1). Stock solutions (1 g L^{-1}) of each pesticide standard were prepared by dissolving the weighed amount in acetone (J. T. Baker, Deventer, Holland), except dimethenamid which was dissolved in ethanol (J. T. Baker, Deventer, Holland). The solutions were stored at −18 °C. Working standard mixed solutions (10 mg L^{-1} and 1 mg L^{-1} of each compound) were prepared weakly by diluting the individual stock solution with acetone and storing at 4 °C. Water standard solutions (25 μg L^{-1}) were used for optimizing the SPME method. Highly purified deionized water (Purelab Option-R7, Elga, UK) was used to dilute the mixed acetone solutions. Sodium chloride (99.5% purity) was purchased from Merck (Darmstadt, Germany) and hexane, acetonitrile and methanol from J. T. Baker (Deventer, Holland).

The fibres used (Supelco, Bellefonte, PA, USA) were: 100 μm polydimethyl-siloxane (PDMS) and 85 μm polyacrilate (PA). Before use, the fibre was conditioned in a gas chromatograph injection port as recommended by the manufacturer. A magnetic stirrer (Roth RCT Basic, Germany) and 8×3 mm stirring bars were used to mix the samples during extraction. Extraction was performed in 4 mL vials (Supelco).

Three samples of uncontaminated Serbian soils originating from Kosjerić (soil A), Kikinda (soil B) and Bela Palanka (soil C) were used in the study. The main physico-chemical properties of these soils are given in Table 2. The soils were air dried and sieved (2 mm) before using.

Polypropylene centrifuge tubes with caps (50 mL) (Sarstedt, Germany), filter papers 1PS, 150 mm diameter (Watman Int. Ltd., Maidstone, UK) and a centrifuge (UZ 4, Iskra, Slovenia) were used in the soil extraction procedure.

Instrumentation

A gas chromatograph-mass spectrometer (GC-MS) was used as a detection device (CP-3800/Saturn 2200, Varian, Australia). A 30 m × 0.25 mm × 0.25 μm, VF-5ms column (Varian) was used. The GC was programmed as follows: initial temperature was 120 °C, then increased to 170 °C at 8 °C min^{-1} and held for 4.5 min, increased to 280 °C at 9 °C min^{-1} and held for 5.5 min. Helium was used as the carrier gas and its flow rate was 1.1 mL min^{-1}.

The ion trap mass spectrometer was operated in the electron impact/selected ion monitoring (EI/SIM) mode. The ion trap and transferline temperatures were set to 220 and 250 °C, respectively. One specific pesticide ion was selected for detection and quantification, while a second one was used for confirmation. The ions inspected are presented in Table 1.

Optimization of DM-SPME analysis

DM-SPME conditions, such as the fibre type, desorption temperature and time, extraction time and NaCl content, were investigated and optimized using 4 mL of aqueous solution containing 25 μg L^{-1} of each pesticide.

Table 1. Physico-chemical properties of pesticides studied,[a] their characteristic quantitative (qualitative) *m/z* ions and optimal desorption temperatures (T_{opt}) and times (t_{opt}) for PDMS and PA fibres

Pesticide	Chemical class	M_r [b]/ (g mol^{-1})	Water Solubility/ (mg L^{-1})	log K_{ow} [c]	H [d]/ (Pam^3 mol^{-1})	Ions monitored/ (*m/z*)	T_{opt}		t_{opt}		PDMS$_{opt}$ / PA$^f_{opt}$
							PDMS	PA	PDMS	PA	
Atrazine	Triazine	215.7	33	2.5	1.5×10^{-4}	200 (215)	270	275	9	7	1.4
Simazine	Triazine	201.7	6.2	2.1	5.6×10^{-5}	201 (186)	275	280	7	9	0.7
Prometryn	Triazine	241.4	33	3.1	1.2×10^{-3}	241 (226)	270	280	7	7	5.3
Phorate	Organophosphorus	260.4	50	3.92	5.9×10^{-1}	231 (121)	275	280	7	7	1.9
Chlorpyrifos	Organophosphorus	350.6	1.4	4.7	6.76×10^{-1}	314 (258)	270	280	7	7	1.3
Fenitrothion	Organophosphorus	277.2	14	3.43	6.65×10^{-2}	260 (277)	275	280	7	7	1.3
Terbufos	Organophosphorus	288.4	4.5	2.77	6.58×10^{-3}	231 (203)	270	285	7	7	1.7
Tebupirimfos	Organophosphorus	318.4	5.5	n. f.[e]	2.89×10^{-1}	318 (261)	275	280	7	5	1.3
Dimethenamid	Chloroacetamide	275.8	1200	2.15	8.32×10^{-3}	154 (230)	270	275	7	9	3.3
Acetochlor	Chloroacetamide	269.8	223	4.14	3.83×10^{-1}	223 (146)	270	275	7	7	2.9
Prochloraz	Imidazole	376.7	34.4	4.12	1.64×10^{-3}	308 (266)	270	280	7	7	2.2
Tebuconazole	Triazole	307.8	36	3.7	1×10^{-5}	250 (125)	275	280	9	9	0.9
Metribuzin	Triazinone	214.3	1050	1.6	1×10^{-5}	198 (215)	270	280	5	7	0.8
Chloridazon	Pyridazinone	221.6	340	1.19	$< 6.52 \times 10^{-6}$	221 (77)	270	280	7	9	5.1
Chlorothalonil	Chloronitrile	265.9	0.81	2.92	2.5×10^{-2}	266 (229)	270	275	9	9	1.3
Pendimethalin	Dinitroaniline	281.3	0.3	5.18	2.728×10^{-3}	252 (191)	275	285	9	9	1.2
Oxyfluorfen	Diphenyl ether	361.7	0.116	4.47	9.40×10^{-2}	252 (317)	270	280	7	7	1.2
Clomazone	Isoxayolidinone	239.7	1100	2.5	4.19×10^{-3}	204 (125)	270	275	7	7	1.9
Fluorochloridone	Pyridazinone	312.1	35.1	3.36	3.9×10^{-3}	315 (174)	270	280	7	7	0.7
Lindane	Organochlorine	290.8	8.52	3.5	0.15	183 (219)	270	280	7	5	1.2

[a] Information taken from literature;[11,12] [b] Molecular weight; [c] Partition coefficient between n-octanol and water (as the log value); [d] Henry's constant; [e] Not found; [f] A (PDMS$_{opt}$)/A (PA$_{opt}$), ratio of analytical signals under optimal desorption conditions for PDMS and PA fibres.

Table 2. Soil physico-chemical properties

Soil	pH (H_2O)	O. M.* / (%)	Sand / (%)	Silt / (%)	Clay / (%)
A	6.72	3.43	28.40	47.96	23.64
B	8.39	3.17	73.96	22.60	3.44
C	7.54	8.69	52.08	24.48	23.44

*Content of organic matter.

In order to determine optimum desorption temperature and time, half-hour extraction procedures were performed at ambient temperature. In the first set of experiments, desorption temperature was varied from 265 to 285 °C with desorption time of 5 min. After that, desorption time was varied from 5 to 9 min at the chosen optimal desorption temperature.

In order to determine optimum extraction time and NaCl content, we also determined the effect of microextraction time from 10 to 60 min, *i.e.* the effect of NaCl content from 0 to 15% (m/v) on the efficiency of SPME.

The following SPME conditions were found to be the most efficient for simultaneous extraction of the selected pesticides: 100 μm PDMS fibre, desorption for 7 min at 270 °C, extraction time of 30 min, 5% NaCl content (m/v).

Soil extraction optimization

Efficiency of the method optimized for SPME of aqueous solutions was tested in the analysis of soil samples. In this part of the study, sub-samples of 8 g (soil B) were placed in polypropylene centrifuge tubes and fortified at 30 μg kg^{-1} level of each pesticide using 1 mg L^{-1} mixed standard solution. The spiked samples were homogenized for 15 min using a rotary stirrer and left to rest for 24 h prior to further analysis.

The extraction efficiencies of various solvents (water, hexane, acetonitrile, acetone and methanol) and the optimum number of extraction steps were determined by the following procedure: soil samples were extracted with 15 mL of solvent for 30 min using a rotary stirrer and then centrifuged for 15 min at 4000 rpm. The extract was filtered and evaporated to dryness at 35 °C using a rotary evaporator. As all analyzed pesticides have good solubility in acetone,[11] dry residues were redisolved in 1 mL acetone, and 0.2 mL of these solutions were diluted with water to 10 mL for DM-SPME measurements. In that way, it was ensured that the presence of organic solvent (2%) did not affect SPME measurements and the fibre life.[10,13-16]

Finally, in order to determine whether and how much the different soil properties effected SPME efficiency, the optimized liquid-solid extraction procedure followed by SPME measurement was applied to analyse the selected pesticides in three different soil types.

Results and Discussion

DM-SPME optimization

Different experimental parameters that affect SPME measurements were optimized using spiked water samples. Optimization was done by a well-structured step-by-step approach including the choice of a most suitable SPME fibre, determination of optimal desorption temperature and time, extraction time and NaCl content.

Fibre selection and optimization of desorption conditions

Because polydimethyl-siloxane (PDMS) and a polyacrylate (PA) fibres have been most throughly studied and usually described as more efficient in pesticide extraction,[5,7,17,18] these fibres were chosen for our study. Table 1 shows optimal desorption temperatures for all pesticides and both fibres used in the experiment. As the best analytical signals for most analytes were recorded at 280 °C for the PA fibre, and 270 °C for PDMS, these temperatures were identified as optimal for the PA and PDMS sorbents.

Table 1 also shows optimal desorption times for all pesticides and both fibres used in the experiment. The results suggest that 7 min can be chosen as optimal desorption time for both fibres. The table also shows the ratios of analytical signals under optimal desorption conditions for both fibres. The data indicate that the PDMS fibre is considerably more efficient for most pesticides (except simazine, metribuzin fluorochloridone and tebuconazole), which is why it was chosen for further work.

Between two measurements, desorption of a blank fibre was done to ensure that no residual compound was present on the fibre.

Optimization of extraction time

Time dependence of the amount of analytes extracted by the fibre was investigated at intervals ranging from 10 to 60 min. The results indicate that for some analytes (phorate, terbufos, tebupirimfos, fenitrothion, chlorpyrifos, pendimethalin, oxyfluorfen, chloridazon, tebuconazole and prochloraz) this interval was not enough to overtake the sorption equilibrium. This is in line with the well established fact that high molecular weight compounds, due to their low diffusion, and compounds that have higher affinity toward the SPME fibre need longer extraction times to overtake equilibrium.[19,20] Considering the pesticides' molecular weights, water solubility and log K_{ow} shown in Table 1, it is evident that our results are in accordance with the rules mentioned.

Although extraction using equilibrium time is recommended, some theoretical models proposed for explanation of the SPME process have indicate that quantification is possible before a sorption equilibrium is reached,[21-23] so that a 30 min extraction time, for practical reasons, was chosen in the following experiments. The time period of 30 min has been found enough time to provide sufficient analytical sensitivity for all compounds studied. In addition, this interval was in accordance with the chromatographic run time (in our case 28.47 min), which ensured a maximum sample throughput when manual extraction was applied.

Effect of ionic strength

An addition of salt to a sample would decrease the solubility of some analytes in the aqueous phase, which stimulates their movement into the fibre coating.[19] For that reason, the effect of ionic strength on the SPME process was studied by adding different amounts of NaCl to the water mixed standard solutions (0, 2.5, 5, 10 and 15% (m/v)).

The results indicate that ionic strenght affects SPME efficiency in different ways and that the yield of SPME depends on the nature of each pesticide. Thus, on the basis of compounds behaviour, considering their logK_{ow} values and solubility (Table 1), they can be classified in three groups. The first group includes compounds whose extraction efficiencies decrease as the percentage of NaCl added to the solution increases. This group consists of more hydrophobic pesticides such as chlorpyrifos, pendimethalin and oxyfluorfen, which have log K_{ow} values higher than 4

and water solubility equal or less than 1.4 mg L^{-1} (Table 1). Some compounds with intermediate polarity, like terbufos and tebupirimfos, were also included in that group. This dependence type is characteristic of chloridazon too, which is unexpected because of its higher polarity (log K_{ow} = 1.19 and solubility 340 mg L^{-1}). A possible explanation for this behaviour of chloridazon is the fact that strong competition between this pesticide and the other ones for PDMS fibre can occur when analyzing 20 compounds in one sample. This can result in a decrease in extraction efficiency for chloridazon. The second group includes pesticides for which the extraction yield increased with increasing ionic strength until a certain value was reached, and then it decreased. An explanation of this behaviour can be the intermediate polarity of these compounds. Polar compounds are known to have a low affinity for the PDMS fibre, which can be increased by decreasing their solubility in water through an addition of NaCl or some other salt. We may assume that these "intermediate" compounds behave as polar until a specific concentration of NaCl in the solution is reached. After that, strong competition between these compounds and the more polar ones for the PDMS fibre can occur, resulting in decreasing extraction yield. The second group includes phorate, prochloraz and metribuzin. The log K_{ow} values and solubility of the first two analytes are 3.92 and 4.12, *i.e.* 50 and 34.4 mg L^{-1}, respectively. As metribuzin is a more polar compounds than the other two (log K_{ow} = 1.6 and solubility 1050 mg L^{-1}), it was unexpected that it should belong to this group of pesticides. As for chloridazon, an explanation of its behaviour could be the presence of strong competition between this analyte and the other ones for sorption on the PDMS fibre. The third group of compounds is made up of polar pesticides and several pesticides of intermediate polarity, whose extraction yields increased with the increase of NaCl content. These compounds are characterized by high solubility in water and/or lower log K_{ow} values, as the case is with triazines (atrazine, simazine and prometryn), clomazone, chloroacetamides (dimethenamid and acetochlor), tebuconazole, fenitrothion, chlorothalonil and fluorochloridone. Interestingly, lindane as an organochlorine pesticide was characterized by this dependence type. However, the same result was reported by Zhao *et al.*,[6] who found that SPME efficiency for lindane increased with an addition of NaCl, quite the opposite to other organochlorine pesticides. Figure 1 shows the effect of ionic strength on analytical signals for pendimethalin, prochloraz and dimethenamid as the representative pesticides of each group.

Comparing our results with some other findings, an agreement is evident in the obtained trends for triazines (atrazine and simazine),[9,16] some organophosphorus

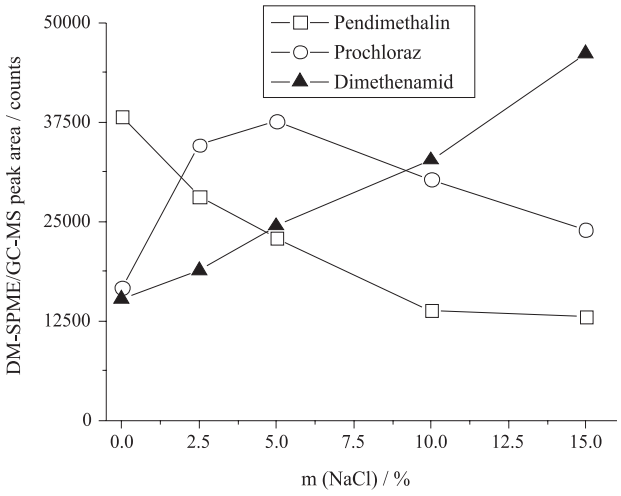

Figure 1. Effect of ionic strength on the analytical signal for pendimethalin, prochloraz and dimethenamid.

pesticides (phorate, fenitrothion[24] and chlorpyrifos[1,8]), chlorothalonil,[17] as well as lindane.[6] However, for some pesticides the trends observed were different from those reported in literature (prochloraz,[25] chlorpyrifos,[24] chlorothalonil,[26] prorate[1] and fenitrothion[1,26]). As mentioned before, during SPME analysis competition may occur among compounds of different polarity for the PDMS fibre. As other authors may also have some pesticides that we have investigated, and additionally some others that we did not examine, it is possible that the intensities of competition between various pesticides were different. This is a possible explanation of the difference between our and their results.

Finally, considering the results obtained for all pesticides in this study, a 5% NaCl content was chosen as optimal.

Soil extraction optimization

Efficiency of the optimized SPME method was checked in an analysis of soil samples. As mentioned before, DM-SPME of a soil organic extract obtained by conventional solid-liquid extraction diluted with an appropriate amount of water was shown to be a more efficient method than immersion of the SPME fibre in the slurry of soil sample and distilled water.[8-10] Therefore the first approach was chosen as the sample preparation step.

Extraction efficiencies of various solvents (water, hexane, acetonitrile, acetone and methanol) and the optimum number of extraction steps were determined by a well-structured step-by-step approach. At first, the choice of a most efficient solvent was made by applying a single extraction procedure. In general, for most of the selected pesticides, the recoveries obtained with methanol were

higher than those with other solvents, and methanol was therefore chosen for further work. The next step was to determine optimum extraction steps. Hence, the extraction of spiked soil samples with methanol was repeated up to four times under the same procedure. For most pesticides studied, the best recoveries were achieved after two extraction steps. For example, Figure 2 presents the results obtained for acetochlor, chlorothalonil and chloridazon. Finally, according to the results obtained in these two sets of experiments, two successive extractions with methanol as the extraction solvent were chosen as the optimal sample preparation procedure.

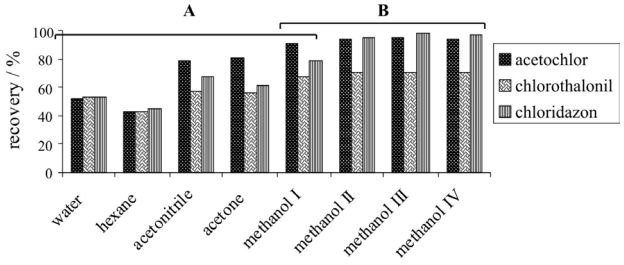

Figure 2. Dependence of extraction efficiency on: A) type of organic solvent and B) number of extraction steps, using the most efficient solvent.

Validation of proposed method

The optimized liquid-solid extraction procedure followed by SPME measurement was applied to analyse the selected pesticides in three different soil types. The main physico-chemical properties of these soils are given in Table 2.

Linearity of the developed method was tested in a concentration range from 2 to 600 μg kg^{-1}. The obtained arrangements and correlation coefficients (R) for all pesticides and soils under study are presented in Table 3. It shows that the correlation coefficients obtained exceeded 0.99 for all compounds exept simazine and soil A (R = 0.988). The somewhat lower correlation coefficient for simazine is probably the results of a slightly lower sensitivity of simazine to the PDMS fibre.

The limit of detection was determined according to IUPAC recommendations.[27,28] LODs were calculated as $3.29 \times s_B$, where s_B is the blank standard deviation.

LODs for all pesticides studied were less than 5 μg kg^{-1}, except for phorate (5.13 μg kg^{-1}, soil A) (Table 4). Precision and confidence of the developed method were determined by performing four consecutive measurements of the soil samples fortified at 30 μg kg^{-1} level. Both, relative standard deviation (RSD) and recovery values are presented in Table 4. The table shows that RSDs for all pesticides and soils under study were below 19%. As RSDs below 20% may be considered acceptable[29] in trace analysis, the proposed method can be satisfactory in terms of precision. On the other hand, our values are consistent with those reported by other authors.[2,3,5,8]

Table 3. Linearity ranges (μg kg^{-1}) and correlation coefficients (R) for pesticides and soils under study

	Soil A		Soil B		Soil C	
	Concentration range / (μg kg^{-1})	R	Concentration range / (μg kg^{-1})	R	Concentration range / (μg kg^{-1})	R
Atrazine	2-600	0.999	2-600	0.996	2-600	0.999
Simazine	10-400	0.988	10-600	0.999	10-400	0.996
Prometryn	2-600	0.998	2-600	0.995	10-600	0.994
Phorate	10-600	0.999	10-600	0.999	10-600	0.996
Chlorpyrifos	2-600	0.997	2-600	0.994	2-600	0.999
Fenitrothion	2-600	0.999	2-600	0.999	2-600	0.993
Terbufos	2-600	0.997	2-600	0.991	2-600	0.991
Tebupirimfos	2-600	0.999	2-600	0.991	2-600	0.996
Dimethenamid	2-400	0.999	2-600	0.999	2-400	0.997
Acetochlor	2-600	0.999	2-600	0.998	2-600	0.997
Prochloraz	10-600	0.996	10-600	0.996	10-600	0.998
Tebuconazole	2-600	0.998	2-600	0.996	10-600	0.992
Metribuzin	10-600	0.995	2-600	0.999	2-600	0.999
Chloridazon	10-600	0.998	2-600	0.995	10-600	0.995
Chlorothalonil	10-400	0.994	10-400	0.998	10-600	0.999
Pendimethalin	2-600	0.999	2-600	0.999	2-600	0.996
Oxyfluorfen	2-600	0.998	2-600	0.999	2-600	0.999
Clomazone	2-600	0.997	2-600	0.999	2-600	0.999
Fluorochloridone	2-600	0.999	2-600	0.998	2-600	0.998
Lindane	2-600	0.994	2-600	0.994	2-600	0.999

Table 4. Recoveries (%, n = 4), relative standard deviations (RSDs, %) and limits of detection (LODs, μg kg^{-1}) obtained for pesticides and soils under study

	Soil A			Soil B			Soil C		
	Recovery / (%)	RSD / (%)	LOD / (μg kg^{-1})	Recovery / (%)	RSD / (%)	LOD / (μg kg^{-1})	Recovery / (%)	RSD / (%)	LOD / (μg kg^{-1})
Atrazine	94.30	6.27	1.65	89.93	9.45	0.72	90.89	5.33	0.95
Simazine	85.57	16.30	3.42	75.76	18.23	4.15	77.27	11.47	4.44
Prometryn	72.43	10.85	0.95	74.56	16.97	0.72	68.94	10.22	2.68
Phorate	33.06	18.55	5.13	36.61	17.30	4.25	41.76	13.09	4.62
Chlorpyrifos	57.30	18.79	1.58	50.25	16.62	1.52	55.37	9.93	1.73
Fenitrothion	69.04	7.85	0.11	71.57	15.06	0.08	68.36	10.40	0.17
Terbufos	50.88	10.81	1.82	54.83	4.42	0.81	51.80	6.75	1.60
Tebupirimfos	63.99	14.86	0.07	60.30	16.18	0.08	53.38	5.32	0.09
Dimethenamid	85.28	17.09	0.45	76.21	18.46	1.97	84.90	17.58	1.23
Acetochlor	84.02	2.22	0.27	93.77	7.79	0.26	89.32	9.80	0.18
Prochloraz	53.67	14.67	3.95	55.82	16.39	2.77	49.56	16.96	3.83
Tebuconazole	72.13	17.13	1.99	75.29	17.42	1.87	67.88	15.34	3.72
Metribuzin	99.58	11.54	2.34	103.50	7.35	0.70	101.04	12.61	1.35
Chloridazon	92.79	14.11	2.17	95.26	11.01	1.01	79.16	14.02	3.02
Chlorothalonil	81.82	13.51	2.86	70.56	8.52	2.29	78.75	14.51	3.15
Pendimethalin	52.24	17.50	1.83	51.66	18.56	1.72	49.23	16.14	2.57
Oxyfluorfen	67.23	16.70	0.32	62.82	19.20	0.53	56.64	7.95	0.81
Clomazone	84.30	4.28	0.71	89.87	4.31	0.07	90.10	1.68	0.21
Fluorochloridone	73.47	11.29	0.89	74.03	15.08	0.71	70.98	7.52	1.25
Lindane	51.64	2.36	1.13	56.81	8.80	0.48	53.51	5.22	0.82

For most of the analyzed pesticides, the recovery values were higher than 65%. Considering the exceptional complexity of the soil matrix and the fact that the samples were fortified with pesticides and left to rest for 24 h prior to analysis (intending to better simulate real-life conditions), the recovery values of *ca.* 65% may be accepted as satisfactory. On the other hand, these values are consistent with those reported by other authors.[5,8,10] An explanation of the lower recoveries obtained for phorate, terbufos, lindane, tebupirimfos, pendimethalin, chlorpyrifos, prochloraz and oxyfluorfen (soils B and C) can be the strong influence of soil matrix on those pesticides and/or the unsufficient power of methanol as extraction solvent in the sample preparation step. Having all this in mind, a method improvement is surely needed to make it more applicable to the analysis of genuine samples.

In order to determine matrix effects on the developed method, three different soils were chosen. As organic matter and clay mostly participate in the sorption of pesticides in soil,[30] soils with different organic matter and clay contents were chosen. Table 2 shows that soil C has similar clay and higher organic matter content than soil A. It was therefore assumed that the effect of organic content on recoveries can be determined. On the other hand, soil A has similar organic matter content as soil B, but also a higher clay content. Considering the recoveries obtained for these two soils, we were able to determine the effect of clay on the efficiency of the proposed method. However, considering the recoveries (Table 4) and precision of measurements (standard deviation values are not shown), the soils with different physico-chemical properties were not found to have effect on the recoveries. This conclusion was not surprising because similar results had been reported by Bouaid *et al.*[8] However, considering the dependences of the extracted pesticide amount as a function of pesticide concentration in different soil samples, it was noticed that the matrix effects of the soils studied were different. For example, Figure 3 shows the matrix effects of different soils on fenitrothion determination by the proposed SPME method. It shows that the sorption of this pesticide in different soils is similar at lower concentrations, which explains the initial conclusion in which only recoveries (fortification of 30 μg kg^{-1}) had been considered. However, it is obvious that different soil properties do affect the efficiency of the method at higher concentration levels.

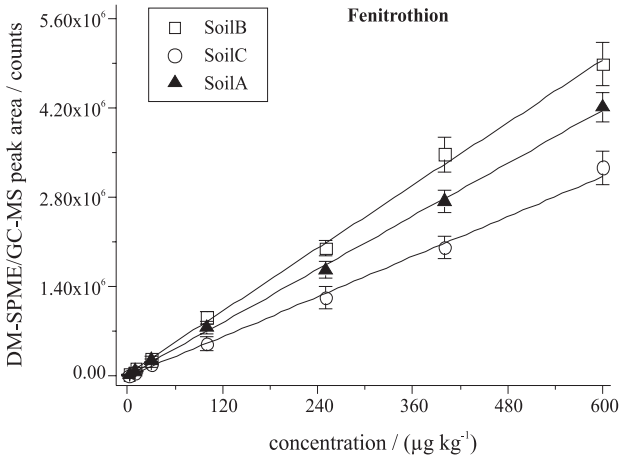

Figure 3. Matrix effect of soils with different physico-chemical properties on fenitrothion determination by the proposed SPME method.

Finally, in terms of linear dependency of all pesticides and soils under study, the pesticides can be classified in five groups. The first group includes compounds whose sorption decreased (recovery increased) in the following order: soil C > soil A > soil B. In this case, both organic matter and clay participated in the pesticides soil sorption. Hence, soil C with high organic matter and clay content sorbed pesticides stronger than soils A and B. On the other side, soil A, which had higher clay content than soil B, sorbed these pesticides stronger than soil B. This group includes fluorochloridone, fenitrothion, tebuconazole, chloridazon, prometryn and prochloraz.

The second group consists of compounds whose sorption decreased in the order: soil A > soil C > soil B (metribuzin, lindane and terbufos). Sorption of these analytes was found to depend primarily on clay content in the soil, *i.e.* sorption was higher when clay content in the soil was higher. Therefore, soil B, which had the lowest clay content (and organic matter), sorbed those compounds more weakly than the other two soils that had higher clay contents. On the other side, the results indicate that soil C sorbed more weakly than soil A. A possible explanation of these results may be that organic matter in soil C had partially covered clay particles and so produced a limited number of active clay sorption sites of that soil.

The third pesticide group includes compounds whose sorption to soil was mainly determined by organic matter content in the soil. For these compounds sorption decreased in the order: soil C > soil B > soil A. Thus, soil C with higher organic matter content than the other two soils sorbed pesticides stronger. In soil A, a part of the organic matter was probably blocked by clay particles that produced a weaker sorption of analytes by the organic surface than soil B. This group consists of chlorpyrifos, tebupirimfos, pendimethalin and oxyfluorfen.

The fourth group includes only acetochlor. The results indicate that sorption of that pesticide by soils was primarily determined by clay content in the soils. Therefore, soil A with high clay and low organic matter content was the strongest sorbent. In soil C, a part of clay sorption sites

Table 5. Concentration limits (μg kg^{-1}) with no significant statistical difference ($p > 0.05$) in sorption between the 2 soil types compared

	Soils A and B	Soils A and C	Soils B and C
Atrazine	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 250 μg kg^{-1}
Simazine	at 10 and 100 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 400 μg kg^{-1}
Prometryn	≤ 100 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 30 μg kg^{-1}
Phorate	≤ 100 μg kg^{-1}	≤ 100 μg kg	≤ 100 μg kg^{-1}
Chlorpyrifos	≤ 400 μg kg^{-1}	≤ 100 μg kg	≤ 100 μg kg^{-1}
Fenitrothion	≤ 100 μg kg^{-1}	≤ 100 μg kg	≤ 30 μg kg^{-1}
Terbufos	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 100 μg kg^{-1}
Tebupirimfos	≤ 100 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 250 μg kg^{-1}
Dimethenamid	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 250 μg kg^{-1}
Acetochlor	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 600 μg kg^{-1}
Prochloraz	≤ 100 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 100 μg kg^{-1}
Tebuconazole	≤ 100 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 30 μg kg^{-1}
Metribuzin	≤ 30 μg kg^{-1}	≤ 250 μg kg^{-1}	≤ 600 μg kg^{-1}
Chloridazon	≤ 400 μg kg^{-1}	≤ 250 μg kg^{-1}	≤ 30 μg kg^{-1}
Chlorothalonil	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}
Pendimethalin	≤ 600 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 100 μg kg^{-1}
Oxyfluorfen	≤ 250 μg kg^{-1}	≤ 250 μg kg^{-1}	≤ 400 μg kg^{-1}
Clomazone	≤ 30 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 250 μg kg^{-1}
Fluorochloridone	≤ 250 μg kg^{-1}	≤ 100 μg kg^{-1}	≤ 30 μg kg^{-1}
Lindane	≤ 100 μg kg^{-1}	≤ 30 μg kg^{-1}	≤ 400 μg kg^{-1}

was probably blocked by organic matter, finally producing a similar sorption capacity of this soil and soil B.

The fifth pesticide group consists of compounds whose sorption behaviour could not be explained by considering organic matter and clay contents alone. Our results indicate that there are actually two sorption trends: soil B > soil C > soil A (atrazine, simazine, chlorothalonil and dimethenamid) and soil A > soil B > soil C (phorate and clomazone). A possible explanation for the "unexpected" behaviour of these pesticides lies either in the difference in sand and silt contents in the analyzed soils (Table 2) or the different nature/origin of those soils (soils A and B were grassland soils, while soil C originated from forest).

Considering the linear dependency observed and applying a two-factor analysis of variance (Statistika '99 Edition, ANOVA/MANOVA), concentration ranges were determined with no statistically significant difference ($p > 0.05$) in sorption between the two soil types compared. The concentration ranges for all soil combinations (A and B, A and C, B and C) are shown in Table 5.

Generally, considering that for most of the pesticides studied, soils with different physico-chemical properties had different effects on their recovery (especially at higher concentration levels), it seems that a standard addition method would be more suitable for quantitative analysis than external calibration based on the use of standard solutions. However, if uncontaminated soil with physico-chemical properties similar to the analyzed soil sample is available, external calibration may be employed using spiked uncontaminated soil samples. It would help avoid possible errors arising from the influence of the matrix.

Conclusions

A solid phase microextraction (SPME) method for simultaneous determination of 20 pesticides belonging to different pesticide groups in soil samples is presented. The method is based on a combination of conventional liquid-solid procedure and SPME determination of pesticides transferred from soil to aqueous solution, so that the time-consuming clean-up step for organic extracts is eliminated. Various microextraction conditions, such as the fibre type, desorption temperature and time, extraction time and NaCl content, as well as the extraction efficiencies of several solvents and the optimum number of extraction steps within the sample preparation step were investigated and optimized. In order to determine matrix effect on the developed method, *i.e.* whether and how much the different soil properties affected SPME efficiency, an optimized liquid-solid extraction procedure followed by SPME measurement was applied to analyse the selected pesticides in three different soil types. The results indicate that soils with different physico-chemical properties have different effects on the recoveries of most pesticides, especially at higher concentration levels. It seems that for that reason, a standard addition method would be more suitable for quantitative analysis than external calibration based on the use of standard solutions. In the situation when uncontaminated soil with physico-chemical properties similar to the analyzed soil is available, quantification using spiked uncontaminated soil samples may be done. In that way, possible errors influenced from the matrix would be avoid.

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