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Determination of Organophosphorus Pesticides in Water, Soil and Biological Samples by Solid-Phase Microextraction and Gas Chromatography

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This paper describes a capillary gas chromatography method for the determination of organophosphorus pesticides in water, soil and biological samples with nitrogen phosphorus detector. The determination of the pesticides (co-ral, DDVP, disyston, ethion, phosdrin, malathion) in samples of tobacco, tea and water with a manual SPME-CG holder using a 100 μm PDMS microfiber, is simple, easy to handle and solvent-free. The optimised conditions for pesticides extraction by SPME-CG method were optimized. Under choise conditions, the analytical curves were linear in different ranges (depend of each pesticide) with correlation coefficients from 0.9987 to 0.9992 and the precision was good (RSD from 3.2 to 7.6 %). The detection limit was 0.004 to 1.800 μg L^{-1} and the quantification limit was 0.009 to 2.500 μg L^{-1}. This method was employed to detect and quantify pesticides with good results.

Key Words: Organophosphorus pesticides, SPME-CG, Water, Soil and biological samples.

INTRODUCTION

The world-wide consumption of organophosphorus pesticides in agricultural activities has increased due to their low persistence in the environment, because they are easily degraded to less harmful compounds and because they are not liposoluble like the organochlorines[1]. The indiscriminate use of organophosphorus pesticides in agriculture has caused environmental problems such as water, soil and vegetable contamination[2,3]. Therefore, the determination of organophosphorus pesticides is received more and more attentions.

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Analysis of pesticides residues in water, soil and biological samples can be performed through gas chromatography (GC) with nitrogen phosphorus detector (NPD)[3], mass selective detector (MSD)[3-6], Electron-capture detector (ECD)[7,8] and high performance liquid chromatography with UV detector (HPLC-UV)[8] with different extraction methods (such as liquid- liquid extraction, Soxhlet extraction, solid phase extraction, solid phase micro-extraction and the like).

This work proposes a solid-phase microextraction (SPME-CG) method to assay organophosphorus pesticide of co-ral (O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)phosphorothioate), DDVP (2,2-dichloroethenyldimethylphosphate), disyston (O,O-diethyl S-[2-(ethylthio) ethyl]phosphorodithioate), ethion (O,O,O',O'-tetraethyl S,S'-methylene *bis*(phosphorodithioate)), phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate), phosdrin (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate), guthion (O,O-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]phosphorodithioate)), malathion (diethyl(dimethoxy thiophosphorylthio succinate) and methyl-parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate) in water, soil and biological samples using gas chromatography with nitrogen-phosphorus detection.

EXPERIMENTAL

The pure standard and the standards solutions of the organophosphorus pesticides were conserved on the freezer in a temperature of 3 to 6 ºC. The stock solution of each pesticide was prepared with mass of 5.0-30.0 mg diluted in 2.0 mL of methanol (Merck, Darmstadt, Germany). The working solutions were performed with dilutions of the stock solutions in water purified by Milli-Q system, (Millipore, 909 A MA, USA). The solvents used were of analytical grade.

Pesticides used as standard were: co-ral (99.4 %), DDVP (93 %), di-syston (98 %), ethion (95 %), phorate (90.6 %), phosdrin (97.2 %), guthion (99.2 %), malathion (91 %) and methyl-parathion (99 %), acquired from PolyScience, Niles, USA.

The chromatographic system used was a HP 6890 gas chromatograph equipped with nitrogen-phosphorus detector (NPD) and an autosampler (G 2613A) (Agilent Technologies, Avondale, PA, USA). A HP-5 capillary column of 30 m × 0.32 mm × 0.25 mm film thickness (Hewlett Packard Company, Avondale, PA, USA). The split/splitless injector was used in splitless mode at 240 ºC for 5 min. The oven temperature was programmed from 80 ºC held for 1 min, 30 ºC min^{-1} up to 180 ºC held for 50 min and finally 20 ºC min^{-1} up to 280 ºC held for 4 min. The detector used was a nitrogen phosphorus (NPD) with temperature set at 290 ºC. The gas carrier used was helium at a flow-rate of 0.8 mL min^{-1}.

Sample preparation: For soil and biological samples, an amount of 1.00 g of samples was placed in a 20.0 mL headspace vial (Supelco) with addition of 16.0 mL Milli-Q water, which was immediately sealed with Teflon-lined rubber septum-aluminum caps.

For water samples, an aliquot of 16.0 mL of water was introduced into 20 mL Pyrex vials, which were immediately sealed with Teflon lined rubber septum aluminium caps to be analyzed through SPME-CG.

Solid-phase microextraction technique (SPME-CG) was performed with a manual holder and 100 μm thickness polydimethylsiloxane (PDMS) fiber film, assemblies were purchased from Supelco (Bellefonte, PA, USA). The fiber was conditioned with injector temperature of 250 ºC for 40 min and with the immersion of the fiber in a solution of 3 drops of methanol in water at 50 ºC, under stirring of 40 min. After 40 min, the fiber was inserted into the GC injector for 2 h at 250 ºC. A blank of the SPME-CG fiber was carried out before each samples analysis to check memory effect and also to condition the SPME-CG fiber for the next sample.

The glass vial containing the sample with Teflon magnetic stirring bars was put on a vial aluminum rack in a stirrer/heater. The fiber was immersed directly into the sample for 40 min at 30 ºC. After the extraction, it was retreated into the needle and inserted into the GC injector at 240 ºC for thermal desorption and analysis.

The repeatability test was determined by extracting and injecting 13 times the standard aqueous mixture with the following concentrations: co-ral = 10.0 μg L^{-1}; DDVP = 8.0 μg L^{-1}; di-syston = 0.1 μg L^{-1}; phorate = 0.12 μg L^{-1}; phosdrin = 120 μg L^{-1} and malathion = 8.0 μg L^{-1}. Chromatograms of a standard solution of the organophosphorus pesticides, are shown in Fig. 1.

RESULTS AND DISCUSSION

For this work, some SPME-CG parameters were examined and researched. Extractions were performed at room temperature according to Silva[9], Beltran[10], Lambropoulou[11], Tombesi[12]. Since SPME-CG extraction is an exothermic process[13], consequently, by decreasing the temperature, the constant of distribution and the equilibrium efficiency increases.

The polymeric phase of the fiber chosen was PDMS, since several reports[9,11,14-18] of the efficiency of pesticide extractions with this fiber.

An extraction time optimization study was done using a 3.00 mg L^{-1} standard mixture of the following pesticides: co-ral, DDVP, di-syston, ethion, phorate, phosdrin, guthion, malathion and methyl-parathion, at room temperature under stirring.

According to Silva and Cardeal[9], a 2.0 cm needle and a 16.0 mL solution in 20.0 mL (headspace) vials were used.

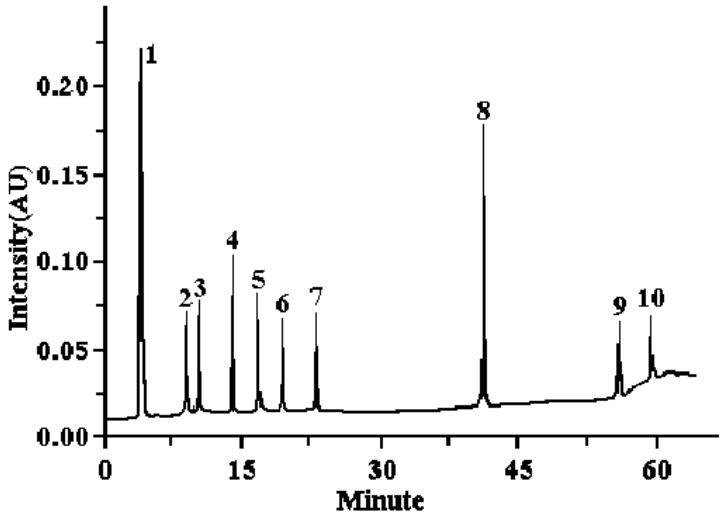

Fig. 1. Chromatograms of standard solution of organophosphorus pesticides and samples. 1 Methanol (2.3 min); 2 DDVP (5.2 min); 3 Phosdrin (6.4 min); 4 Phorate (9.8 min); 5 Di-Syston (12.4 min); 6 Methyl-Parathion (15.2 min); 7 Malathion (18.7 min); 8 Ethion (48.6 min); 9 Guthion (59.8 min); 10 Co-Ral (61.5 min)

For the optimization of the extraction time, absorption times of 25, 40 and 60 min were tested. As shown in Fig. 2, the signal area increased to 40 min for co-ral, ethion, malathion and methyl-parathion pesticides. After this period no significant alteration occurred. Apparently, methyl-parathion and malathion had a good increase in the signal area by raising the time for over 40 min, but as it can be observed in the scale, it is not of great significance. For guthion, phorate and di-syston, times superior to 40 min improved the extraction of pesticides analyzed, while DDVP extractions had no considerable alterations in the extraction times tested. The phosdrin is not included in the Fig. 2 because it was not possible to detect it in a solution of 3.00 μg L^{-1} that is the concentration used in optimization.

Therefore, the time of 40 min chosen for extraction presented a good relationship between the peak areas and an acceptable time of analyses. According to Yao *et al.*[14] in routine analysis, it is not necessary to reach equilibrium, but, the immersion time, stirring and position of the fiber in the solute have to be carefully controlled and kept consistent throughout the experiment.

The desorption time was determined experimentally in 5, 6, 7, 8 and 10 min, keeping constant other optimized parameters of SPME-CG and injector temperature at 240 ºC. It was observed that analytes were desorbed within 10 min of fiber exposure in the injector. This period was then chosen for desorption of the analytes, since it avoided carryover effect.

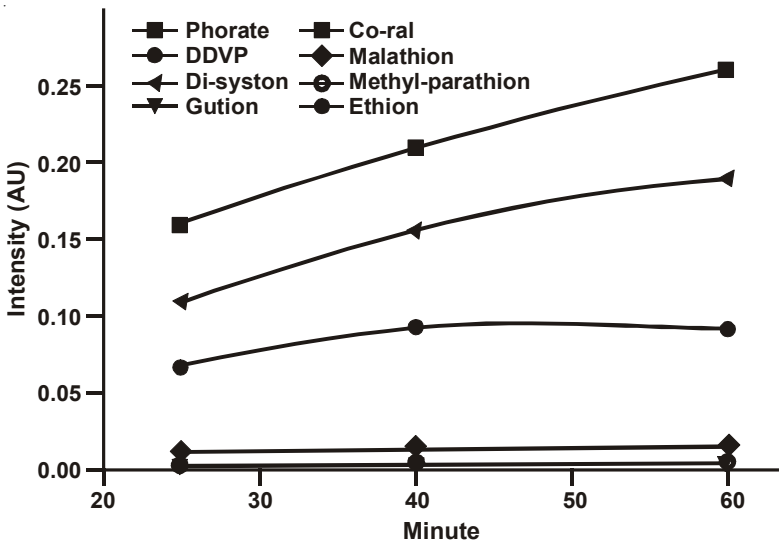

Fig. 2. Time extraction/absorption study of organophosphorus pesticides in a solution of 3.00 μg L^{-1} by a PDMS fiber (extraction at room temperature). Each result represents the mean of three independent experiments

A mixture with different concentrations was necessary for the statistical analysis, since the pesticides presented quite different detections. For the linearity study, standard mixtures in water of organophosphorus pesticides were used in the following range of concentrations: 0.03 to 1.20 μg L^{-1} for phorate and di-syston; 0.8 to 62 μg L^{-1} for co-ral; 0.6 to 56 μg L^{-1} for malathion and DDVP; 8.0 to 485 μg L^{-1} for phosdrin. The pesticides ethion, guthion and methyl-parathion are not represented due to they were not been found in anyone of the samples analyzed.

Regression equations and correlation coefficients were calculated for each pesticide presented in Table-1. It can be observed from the values of correlation coefficients that the equations have good linearity in the range of concentration studied and thus it is possible to quantify these pesticides.

Variance analysis[19] of each pesticide (Table-1) demonstrated that the ratio between the regression average square (MQreg) and the residue average square (MQr) is quite larger than the tabulated Test $F_{1,n-2}$ values in which 1 and n-2 are the numbers of the degree of freedom of the square average due to the regression and the residual quadratic average, respectively, with confidence level of 95 %. This way, regressions are statistically significant.

TABLE-1
LINEAR REGRESSION ANALYSIS PARAMETERS OF ORGANOPHOSPHORUS PESTICIDES

Compounds	Range of concentration (μg L^{-1})	Regression equation	Correlation coefficient (r)
Co-ral	0.80-62.0	Y = 1450X – 628	0.9990
DDVP	0.60-56.0	Y = 92.64X – 28.56	0.9988
Di-syston	0.03-1.2	Y = 10124X – 287.6	0.9991
Phorate	0.03-1.2	Y = 12435X – 684.8	0.9989
Phosdrin	8.00-485.0	Y = 9.87X – 4.863	0.9992
Malathion	0.60-56.0	Y = 964.5X – 116.8	0.9987

Values of relative standard deviation (RSD %), also known as variation coefficient, were calculated in optimized conditions with the concentrations: 10.0 μg L^{-1} for co-ral, 8.0 μg^{-1} for DDVP, 0.12 μg L^{-1} for phorate, 0.11 μg L^{-1} for di-syston, 8.0 μg L^{-1} for malathion and 120.0 μg L^{-1} for phosdrin. Values lower than 10 % were obtained, except for DDVP, which presented a deviation a little higher, 5.7 %, and di-syston, 7.6 % (Table-2). These values indicate that the method has adequate precision.

TABLE-2
PRECISION OF THE METHOD AND LIMITS OF DETECTION (LOD) AND LIMITS OF QUANTIFICATION (LOQ)

Pesticides	Precision-RSD (%)	LOD (μg L^{-1})	LOQ (μg L^{-1})
Co-ral	4.1	0.150	0.200
DDVP	5.7	0.100	0.140
Di-syston	7.6	0.004	0.009
Phorate	3.8	0.060	0.012
Phosdrin	4.5	1.800	2.500
Malathion	3.2	0.080	0.120

Limits of detection (LOD) and limit of quantification (LOQ) were determined according to IUPAC recommendations[19]. 20 Experimental repetitions were performed for the calculation of the blank standard deviation (sB). The limits of detection and quantification were calculated by 3.29 × sB and 16.67 × sB, respectively. The results obtained are available in Table-2. Beltran *et al.*[10] and Yao *et al.*[14] have analyzed organophosphorus pesticides by SPME-CG with flame photometric detector and with nitrogen and phosphorus detector, respectively. They have founded similar limits of detection,

but results obtained by Eisert *et al.*[20] with atomic emission detector were higher than those found by them. However, in this work, limits of detection varied a lot. For the phorate, for example, the limit of detection determined was 0.006 μg L^{-1}, while Beltran *et al.*[10] found 0.020 μg L^{-1} and Yao *et al.*[14] found 0.200 μg L^{-1}. For malathion, the limit of detection was higher than that found by Yao *et al.*[14] and Beltran *et al.*[10].

Samples analysis: The microextraction solid phase extraction procedure presented for organophosphorus pesticides were applied to water, soil, and biological samples. The results for biological samples were given in Table-3.

TABLE-3
DETERMINATION RESULTS OF THE SAMPLES

Components	Determination results of the samples				
	Tap water (μg L^{-1})	River water (μg L^{-1})	Tea (μg L^{-1})	Tobacco (μg L^{-1})	Rice (μg L^{-1})
Co-ral	ND	4.18 ± 0.14	0.16 ± 0.02	0.086 ± 0.2	ND
DDVP	ND	3.18 ± 0.12	0.12 ± 0.03	ND	0.22 ± 0.04
Di-syston	ND	0.48 ± 0.04	ND	0.028 ± 0.005	ND
Phorate	ND	0.08 ± 0.02	ND	0.036 ± 0.003	ND
Phosdrin	ND	1.28 ± 3.21	0.62 ± 0.02	ND	0.36 ± 0.02
Malathion	ND	5.18 ± 0.08	ND	0.028 ± 0.003	ND

ND = Not detected.

Conclusion

This work describes an alternative method for analyses of organophosphorus pesticides in water, soil and biological samples, with SPME-CG 100 μm PDMS fiber. The method proposed in this work proved to be suitable for analysis of organophosphorus pesticides in water, soil and biological samples, showing good precision and linearity. Limits of detection ranged from 0.004 to 1.800 μg L^{-1}, depending on the compound. This method presents advantages since it is solvent-free, efficient, low cost and fast. Hence, it is more practical than the conventional extraction methods, and it involves fewer extraction stages compared to other methods.

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