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Article

A Solid-Phase Microextraction Method for the Chromatographic Determination of Organophosphorus Pesticides in Fish, Water, Potatoes, Guava and Coffee

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Este trabalho descreve um método de Microextração em Fase Sólida (SPME-CG) para a determinação de pesticidas organofosforados em amostras de peixes de água doce, água e outros alimentos por cromatografia em fase gasosa com detector de nitrogênio e fósforo. As amostras foram coletadas entre outubro de 2002 e abril de 2003 nos afluentes e subafluentes do rio Paranaíba que abastecem a cidade de Patos de Minas, Minas Gerais, Brasil. A determinação dos pesticidas co-ral (O,O-dietil-O-(3-cloro-4-metil-2-oxo-2H-1-benzopiran-7-il) fosforotioato)), DDVP (2,2-dicloroetenil dimetilfosfato), di-siston (O,O-dietil S-[2-(etiltio) etil] fosforoditioato), etion (O,O,O',O'-tetraetil S,S'-metilenobisfosforoditioato), forato (O,O-dietil-S-etiltiometilfosforoditioato), fosdrin (O,O-dimetil-1-carbometoxi-1-propen-2-il-fosfato), gution (O,O-dimetil-S-(4-oxo-1,2,3-benzotrizina-3-metil) fosforoditioato)), malationa (O,O-dimetil-S-(etil-1,2-dicarboetoxi) fosforoditioato) e parationa metílica (O-dimetil-O-4-nitrofenilfosforotioato) em amostras de peixes, água e outros alimentos, com o procedimento de SPME-CG utilizando uma microfibra de PDMS de 100 μm, é simples, fácil manuseio, econômica e livre de solvente. As condições otimizadas para a extração dos pesticidas com o método SPME-CG foram: amostras sob agitação, absorção à temperatura ambiente durante 40 min, dessorção a 220°C durante 10 min e volume de amostra no frasco de 16,0 mL. Utilizando-se estas condições foram obtidas curvas analíticas lineares em diferentes faixas de concentração (dependendo de cada pesticida) com coeficientes de correlação entre 0,997 a 0,999. A precisão estava adequada com desvios padrão relativos variando de 4,40 a 15,13%. O limite de detecção variou de 0,05 μg L^{-1} a 8,37 μg L^{-1} e o limite de quantificação de 0,09 μg L^{-1} a 8,70 μg L^{-1}. O método foi empregado para detectar e quantificar pesticidas em 24 amostras de peixes de três espécies diferentes e também em água, batatas, goiaba e café. As amostras analisadas mostraram resíduos de seis pesticidas organofosforados diferentes.

This paper describes a Solid Phase Microextraction method (SPME-CG) to the determination of organophosphorus pesticides in samples of fresh-water fish, water, potatoes, guava and coffee by capillary gas chromatography with nitrogen phosphorus detector. The samples were collected from October 2002 to April 2003 in the tributaries and sub-tributaries of the Paranaiba River, which supplies the city of Patos de Minas, Minas Gerais, Brazil. The determination of the pesticides: co-ral (O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) phosphorothioate), DDVP (2,2-dichloroethenyl dimethylphosphate), 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(4H)-yl)methyl] phosphorodithioate)), malathion (diethyl (dimethoxy thiophosphorylthio succinate) and methyl-parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate) in samples of fish, water and others foods 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: sample agitation, absorption at room temperature for 40 min, desorption at 220°C for 10 min, and sample volume in the vial of 16.0 mL. Under these conditions, the analytical curves were linear in different ranges (depend of each pesticide) with correlation coefficients from 0.997 to 0.999 and the precision was good (RSD from 4.40 to 15.13%). The detection limit was 0.05 μg L^{-1} to 8.37 μg L^{-1} and the quantitation limit was 0.09 μg L^{-1} to 8.70 μg L^{-1}. The method was employed to detect and quantify pesticides in 24 fish of three different species and also in water, potatoes, guava and coffee. The samples analyzed showed residues of six different organophosphorus pesticides.

Keywords: organophosphorus pesticides, SPME-CG, fish and others foods, Paranaiba river

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Introduction

The city of Patos de Minas, geographically located in the west of the state of Minas Gerais, in the micro region of Alto Paranaiba, Brazil, is an essentially agricultural region, which includes 25,890 hectares of cultivated land. The most used pesticides in the agriculture and even in the cattle breeding of this region are the organophosphorus pesticides, whose aim is to control and combat plagues that attack crops and animals.

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[1] and because they are not liposoluble like the organochlorines.

The indiscriminate use of organophosphorus pesticides in agriculture has caused environmental problems such as soil and vegetable contamination and, through leaching, contamination of rivers and its temporaries, drinking water, natural surface waters, marine and fresh water organisms[2] and food.[3] Besides that, aquatic life is compromised. Fish contamination by residues of these pesticides have been temerarious, since they are distributed in the local commerce and consumed by riverside populations.

Organophosphorus pesticides, in the nature, are of ecological concern because they are toxic for non-target insects even in low concentrations.[1] The toxicity of these pesticides is mainly in the inhibition of the acetylcholinesterase activity, the enzyme that degrades the neurotransmitter acetylcholine in cholinergic synapses. The inhibition of acetylcholinesterase causes an accumulation of acetylcholine at the nerve synapses and disruption of the nerve function.[4,5] While the metabolism of these compounds in mammals has been well investigated, the metabolism in species of fish has received less attention.[1] However, it has been demonstrated that fish have the capacity to metabolize a variety of compounds, such as pesticides and others environmental contaminants.[6]

Analysis of pesticides residues in fish can be performed through gas chromatography (GC) with nitrogen phosphorus detector (NPD),[3] mass selective detector (MSD),[3,7-10] Electron-capture detector (ECD)[7,9-13] and High-performance liquid chromatography with UV detector (HPLC-UV),[4,12] with different extraction methods.

To the extraction of residual pesticides in fish, Hernandez *et al*.[3] used a liquid-liquid extraction procedure preceded by a clean-up method through a laborious process which requires high cost solvents.

Ayas *et al*.[7] extracted residues of pesticides with Soxhlet system, using hexane as solvent. It is a lengthy process and large amounts of solvents are used.

Riedel *et al*.[11] carried out extraction of pesticides in fish with dichloromethane using a Dionex 2000 system at 100ºC and 2000 psi. Lipids and other interferents were removed from the tissue extracts by an HPLC system. The extraction method, besides demanding toxic solvent, needs to be performed with high pressure and temperature.

The extraction technique that Hiatt[8] used was vacuum distillation with a laborious and difficult system, using low temperatures.

Kitamura and co-workers[2] have been used dichloromethane in large amounts to perform extractions of pesticide in fish. Samples were cut, homogenized and centrifuged to remove solid materials and were extracted again with dichloromethane. This procedure is lengthy, uses large amounts of solvents and requires various stages to prepare the sample, which can cause loss of analyte and experimental errors.

Mormede and Davies,[12] and Manirakiza *et al*.[9] performed their extractions using the Soxhlet system followed by clean-up. The solvent used by the former was methyl tert-butyl ether (MTBE), whereas the latter used 60 mL of a mixture of hexane and acetone 3:1(v/v) in hot extraction for 2 h. Easton *et al*.[10] also used Soxhlet extraction during 16 hours with dichloromethane. This process of extraction is slow with use of toxic solvent.

Yamaguchi and co-workers[13] did the extraction using isohexane as solvent. The extract containing isohexane was concentrated using N_2 flow, and then eluted with diethylether in isohexane. The extraction procedure with solvent was performed in several stages, which facilitated the loss of analyte through handling.

This work proposes a solid-phase microextraction (SPME-CG) method to assay organophosphorus pesticide in fresh water fish using GC with nitrogen-phosphorus detection. The water of the Paranaíba River, its temporaries and sub-temporaries, as well as potatoes and guava and coffee collected in the region located beside the river were also analyzed.

Experimental

Materials

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 in grams of 5.0 – 30.0 mg diluted in 2.0 mL of methanol (Merck, Darmstadt, Germany). The work solutions were performed with dilutions of the stock solutions in water purified by Milli-Q system, (Millipore,

Milford, 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.

Instrumentation

The chromatographic system used was a 3800 Varian gas chromatograph (Walnut Creek, CA, USA) equipped with a Shimadzu C-R6A Chromatopac integrator (Kyoto, Japan) and a HP-5 capillary column of 30 m x 0.32 mm x 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 80ºC held for 1 min, 30 ºC min^{-1} up to 180ºC held for 50 min and finally 20ºC min^{-1} up to 280ºC 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 collection and preparation

Fish. Six fish samples of two different species (*pimelodus maculatus* and *Axtianax spp)* were collected in November and December 2002 in the Paranaiba River and one of its temporaries (Canavial stream) using a stainless steel fishhook. In March 2003 a second fish sampling was performed. This time eight fish of *pimelodus maculatus* species were collected. The third sampling occurred in April, with the capture of ten fish of two different species, *pimelodus maculatus* and *leoporinus reinhardti*. Samples were frozen and stored at -4 ºC in plastic bags. Analyses were performed in triplicates from 2 to 9 days after sampling.

For the analysis an amount of 0.500 g of fish (muscular tissue parts, tail and gills) 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.

*Water***.** Water samples were collected from October 2002 to January 2003 in the Paranaiba River and in six of its tributaries and sub-tributaries, as well as two artesian wells, one which has been located in a coffee culture site for several years, and another artesian near a tomato, pepper, soy bean and other vegetables culture. Sample stations were selected in order to include possible pesticide sources near the city of Patos de Minas.

Figure 1 shows an overview of the Paranaiba River, its tributaries and sub-tributaries, which provides water for the city of Patos de Minas, where fish and water samples were collected.

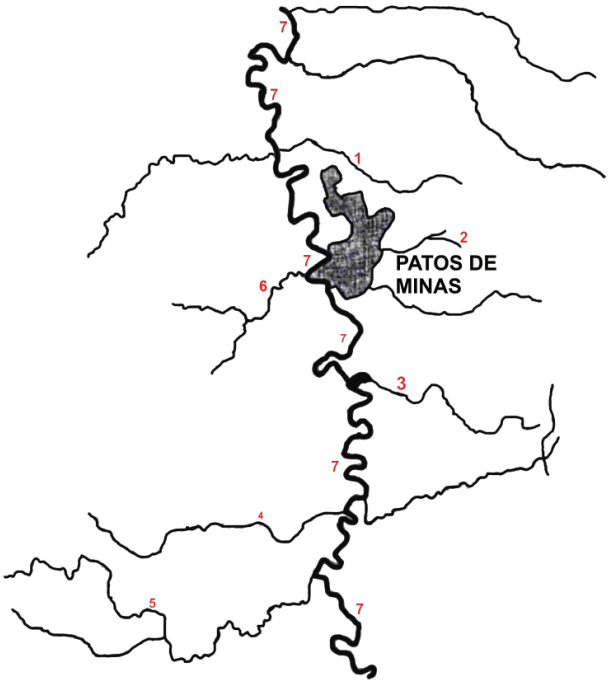

Figure 1. Sample stations for water and fish: 1- Limoeiro stream, 2- Canavial stream, 3- Aragões stream, 4- Contendas stream, 5- Brejo stream, 6- Cota stream, 7- Paranaiba river.

Water samples were collected in amber glass vials with Teflon top and held at the temperature of 3–6 ºC. Analyses were performed in the period of 1 to 8 days after collection. 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.

Fruit, tubercles and coffee. Potato samples were purchased in November and December 2002 in the region of Patos de Minas and were sent to laboratory analysis.

Pieces of pulp and peel of five potatoes were removed in each lot using a stainless steel knife, taking flesh and peel with mass in grams of 0.5218 to 0.6088 that were put, with addition of 16.0 mL of Milli-Q water, 20 mL vials, in 20.0 mL Pyrex vials, sealed with Teflon lined rubber septum aluminum caps.

Guavas samples were similar to those of potatoes, but samples in each analysis were taken from just one fruit for each vial. The mass determined for the guavas was of about 0.5000g.

Samples of coffee grains and leaves were collected in two Patos highway near the tributaries and sub-tributaries of the Paranaiba River. This fruit was prepared like the

other samples, with masses of 0.1805 to 0.1842. All SPME-CG analyzes were done in triplicates.

SPME method

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. Finished this period, the fiber was inserted into the GC injector for 2 hours at 250 ºC. A blank of the SPME-CG fiber was carried out before each sample 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 = 10.03 μg L^{-1}; DDVP = 8.15 μg L^{-1}; di-syston = 0.11 μg L^{-1}; phorate = 0.12 μg L^{-1}; phosdrin = 120.97 μg L^{-1} and malathion = 8.1 μg L^{-1}.

Chromatograms of a standard solution of the organophosphorus pesticides, water, fish, guava and coffee are shown in Figure 2.

Results and Discussion

For this work, some SPME-CG parameters were examined and researched.

Extractions were performed at room temperature according to Beltran,[16] Lambropoulou,[17] Tombesi[18] and Silva,[15] because SPME-CG extraction is an exothermic process.[19] Consequently, by decreasing the temperature, the constant of distribution and the equilibrium efficiency increases.

The polymeric phase of the fiber chosen was PDMS, since literature data bring us several reports,[15,17,20-24] 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.

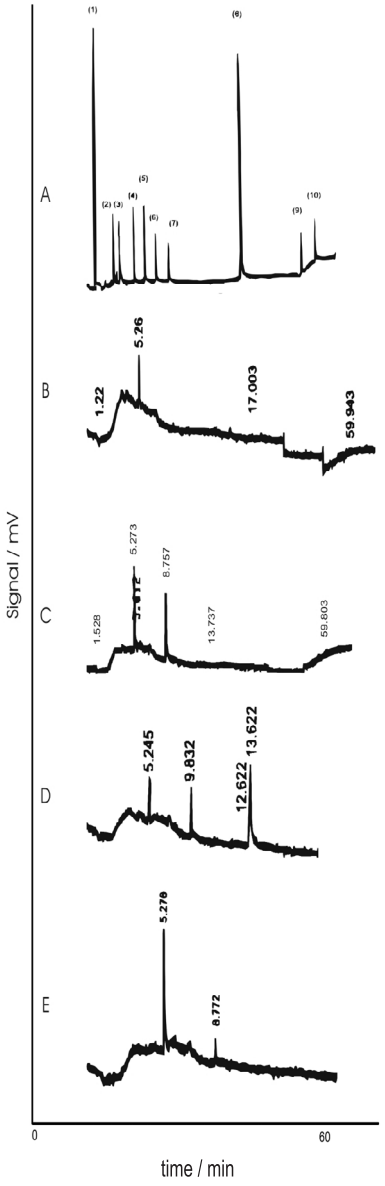

Figure 2. Chromatograms of standard solution of organophosphorus pesticides and samples. A- Chromatogram of a standard solution of the organophosphorus pesticides: 1 Methanol (2.3 min); 2 DDVP (5.2 min); 3 Phosdrin (6.4 min); 4 Phorate (9.8 min); 5 Di-Syston (12.4 min); 6 Methyl-Parathion (15.2 min); 7 Malathion (18.7 min); 8 Ethion (48.6 min); 9 Guthion (59.8 min); 10 Co-Ral (61.5 min). B- Chromatogram of fish samples from Paranaiba River. C- Chromatogram of water sample of Patos de Minas region. D- Chromatogram of Coffee sample. E- Chromatogram of guava sample. Chromatographic conditions in experimental.

According to Silva and Cardeal,[15] a 2.0 cm needle and a 16.0 mL solution in 20.0 mL (headspace) vials were used.

For the optimization of the extraction time, absorption times of 25, 40 and 60 minutes were tested. As shown in Figure 3, 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 Figure 3 because it was not possible to detect it in a solution of 3.00 μg L^{-1} that is the concentration used in optimization.

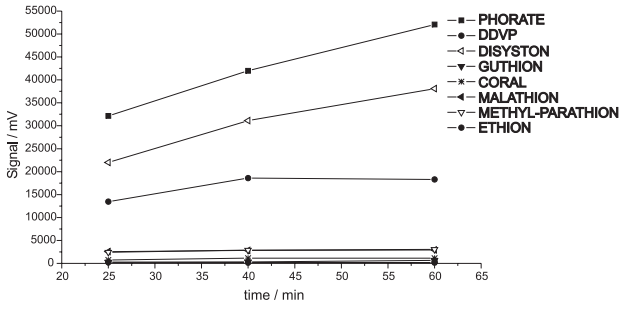

Figure 3. Time extraction / absorption study of organophosphorus pesticides in a solution of 3.00 μg L^{-1} by a PDMS fiber (extraction at room temperature). Each result represents the mean of three independent experiments.

Therefore, the time of 40 min chosen for extraction presented a good relationship between the peak areas and an acceptable time of analyses. Besides, according to Yao *et al.*,[20] 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 all the experiment.

The desorption time was determined experimentally in 5, 6, 7, 8 and 10 minutes, 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.

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 0.47 μg L^{-1} for phorate and di-syston ; 2.61 to 40.12 μg L^{-1} for co-ral; 2.11 to 32.40 μg L^{-1} for malathion and DDVP; 31.45 to 483.88 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 that this way it is possible to quantify these pesticides.

Variance analysis[25] 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.

Values of relative standard deviation (%RSD), also known as variation coefficient, were calculated in optimized conditions with the concentrations: 10.03 μg L^{-1} for co-ral, 8.15 μg L^{-1} for DDVP, 0.12 μg L^{-1} for phorate, 0.11 μg L^{-1} for di-syston, 8.10 μg L^{-1} for malathion and 120.97 μg L^{-1} for phosdrin. Values lower than 10% were obtained, except for DDVP, which presented a deviation a little higher, 11.35%, and di-syston, 15.1% (Table 2). These values indicate that the method has adequate precision.

Limits of detection (LOD) and quantitation (LOQ) were determined according to IUPAC recommendations.[26] Twenty experimental repetitions were performed for the calculation of the blank standard deviation (s_B). The limits of detection and quantitation were calculated by 3.29 x s_B and 16.67 x s_B, respectively. The results obtained are available in Table 2. Yao *et al.*[20] and Beltran *et al.*[16] have analyzed organophosphorus pesticides by SPME-CG with flame photometric detector and with nitrogen and phosphorus detector, respectively. They have founded very similar limits of detection, but results obtained by Eisert *et al.*[27] with atomic emission detector were larger than those found by them. However, in this work, limits of detection

Table 1. Linear regression analysis parameters of organophosphorus pesticides

Compounds	Range of concentrations (μg L^{-1})	Regression Equation	Correlation Coefficient (R)
Co-ral	2.61 – 40.12	Y=1202.89 X – 1441.36	0.998
DDVP	2.11 – 32.40	Y=88.57 X – 80.26	0.997
Di-syston	0.03 – 0.47	Y=9710.65 X – 112.82	0.998
Phorate	0.03 – 0.47	Y=11593.94 X – 139.07	0.998
Phosdrin	31.45 – 483.88	Y=8.29 X – 126.87	0.998
Malathion	2.11 – 32.40	Y=891.79 X – 1266.96	0.997

Table 2. Precision of the method and limits of detection and quantitation

Pesticides	Precision – RSD (%)	Limit of Detection (μg L^{-1})	Limit of Quantitation (μg L^{-1})
Co-ral	8.19	0.482	0.505
DDVP	11.35	0.502	0.807
Di-syston	15.10	0.005	0.009
Phorate	7.57	0.011	0.014
Phosdrin	8.96	8.374	8.691
Malathion	4.41	1.097	1.117

varied a lot. For the phorate, for example, the limit of detection determined was 0.011 μg L^{-1}, while Beltran *et al*.[17] found 0.020 μg L^{-1} and Yao *et al*.[20] found 0.200 μg L^{-1}. For malathion, the limit of detection was higher than that found by Yao *et al*.[20] and Beltran *et al*.[16]

Samples analysis

There are few studies of organophosphorus analysis in fish in comparison with organochlorine pesticides. In spite of organophosphorus pesticides have arisen to replace organochlorine because they are not bio accumulative, they are absorbed in epithelium gills of fish. Its high toxicity indicates that there should be routine analysis in the regions where these pesticides are used.

In samples of *pimedolus maculatus* collected in the Paranaiba River, just after the first spring rain, residues of DDVP were detected with concentration of 0.00010 mg kg^{-1}. DDVP is classified by the Environmental Protection Agency (EPA) as having a toxicity risk index of 1 because it can cause cancer and it is considered as a restricted use pesticide.[28] The DDVP lethal concentration,[29] LC_{50}, in the species of *Lepomis macrochirus*, find in the Mississippi River and known as *bluegills*, is 0.9 mg L^{-1}. In certain species of fish, concentrations of 0.25 - 1.25 mg L^{-1} cause inhibition of acetylcholinesterase activity in the brain and in the liver.[29]

The parts of fish analyzed in this work were: tail, gills, epithelium and dermal tissue, being DDVP present only in the gills. In the other samples of fish collected in the summer and in the beginning of autumn no residue of the pesticides investigated was detected.

The retention time of 5.26 min in the chromatogram of fish (Figure 2) identify the DDVP.

According to regulation number 10 (03/08/1985) of the National Secretary of Sanitary Vigilance[30] updated by the Brazilian Association of Sanitary Vigilance (ABIA – 06/30/1996) the concentration of DDVP allowed in animal products, meat and meat products is 0.05 mg kg^{-1}. This value is confirmed by the Codex Alimentarius.[31] The value obtained in this work was quite below the one stipulated by the agencies mentioned above.

In environmental monitoring, the Acceptable Daily Intake (ADI), which is 0.004 mg kg^{-1} of body weight,[28,31] should be taken into account for the residue of pesticide found in fish. Probably the quantified concentration in fish hardly ever exceeds the ADI.

Rishi and Grewal[32] showed that DDVP absorpted through the gills epithelium affects the chromosomes of *Channa punctatus* and that fish is efficient as a model in the conduction of genotoxic investigations related to water pollutants.

This pesticide can come from agricultural crops and from houses and stores wastes, since it is used to control a variety of insects. The presence of DDVP in fish is also justified because near the Paranaiba River there is a municipal slaughterhouse which contributes with a large amount of pesticides, mainly organophosphorus and especially DDVP, which is widely used by cattle breeders of this region. Wastes such as the slaughterhouse cleaning water are thrown directly into the river, which may be contributing to the rivers water contamination and even fish.

Since fish analyzed has been presented residues of pesticide, one tried to check the spread of the contamination in the region of Patos de Minas, was carried out analysis in water, guava, coffee and potato samples.

Results of triplicate analysis of water of the Paranaiba River tributaries and sub-tributaries are presented in Table 3. Six types of organophosphorus pesticides residues were detected in the samples analyzed. Phorate was present in six out of the eight samples analyzed and DDVP was found in three sampling sites. These two pesticides are widely used in the control of insects and plagues that attack the several crops located in Patos de Minas and DDVP has been widely used in animals, especially cattle. In samples of water collected before the rainy season no kind of pesticide was detected. These results were found in samples collected after the beginning of the rainy season. Waters collected after a long rainy season were analyzed and did not present any residue of the pesticides investigated.

The maximum value permitted in water is 0.1 μg L^{-1} for each pesticide, and 0.5 μg L^{-1} for the total of pesticides, according to the WHO.[15] On the other hand, limits established by the Brazilian Environment National Council

Table 3. Analysis of organophosphorus pesticides in samples of water

Sample Stations	Pesticides Concentrations (μg L^{-1})						
	Co-ral	DDVP	Di-syston	Phorate	Phosdrin	Malathion	Sample number
Aragões $stream_1$	4.42±0.19	3.16±0.14	ND	ND	ND	ND	3
Aragões $stream_2$	ND	ND	0.31±0.05	0.05±0.06	ND	ND	3
Limoeiro $stream_1$	ND	ND	ND	0.04±0.03	242.03±7.69	ND	4
Limoeiro $stream_2$	ND	6.45±0.51	ND	0.02±0.01	ND	ND	4
Canavial stream	ND	ND	0.69±0.04	0.08±0.01	ND	4.93±0.05	4
Cota stream	ND	ND	ND	0.02±0.01	ND	ND	3
Artesian Wel	ND	ND	ND	0.04±0.02	ND	ND	2
Paranaíba River	ND	2.18±0.22	ND	ND	ND	ND	4

N.D = non-detected. Indices 1 and 2 indicate collections analyzed in different dates.

(CONAMA),[34] for guthion is 0.05 μg L^{-1} and for malathion is 0.1 μg L^{-1} and 10.0 μg L^{-1} (that is expressing with concentration of parathion) for the total sum of organophosphorus and carbamates. But as there are cultures that sometimes make use only of carbamates and other times only organophosphorus pesticides, it would not be adequate to use this total sum limit. Legislations do not include all pesticides used. It can be observed that the values found for waters analyzed are quite above the limit established by the WHO. Phosdrin was the pesticide that presented the highest concentration, followed by DDVP, malathion and co-ral. The high concentrations found, mainly for the phosdrin, is due to the places where the samples were collected are been located near to several kinds of crops and the rain water glance over the crops disembogue on the watercourse where the levies were done.

Phorate, the most common, was detected with lower concentration in relation to the others, being its value under the one prescribed by WHO. Lambropolou *et al.*[17] have been detected di-syston in the Kalamas River (Greece) with concentration ranging from 0.015 to 0.025 μg L^{-1} in the period of May to September. Values determined in this work were higher than those they determined for di-syston and those that Zulin *et al.*[35] have been determined for DDVP in the Jiulong River estuary (JLRE – China), whose concentration ranged from 6.67 to 49.8 ng L^{-1}, and those that Zhang[36] has been determined in Pearl River estuary (China), 0.17 to 5.80 ng L^{-1}. The concentrations of malathion determined in waters were higher than those of JLRE, 51.6 ng L^{-1} detected by Zulin *et al.*,[35] higher than those of the Indian estuary (India): 1.373 – 13.013 ng L^{-1}, determined by Sujatha *et al.*,[37] and higher than those of the Humber estuary (England): 1 – 9 ng L^{-1}, detected by Zhou *et al.*[38]

Results of potatoes, guava and coffee analysis are shown in Table 4. It can be observed that all of the samples of potatoes analyzed, as well as fruit and coffee, presented residues of DDVP. Yet, phorate residues are present in grains and leaves of coffee with concentration of 0.02 μg kg^{-1}. The Brazilian National Agency of Sanitary Vigilance (ANVISA),[39] prescribes that the maximum limit permitted for phorate in coffee is 0.05 mg kg^{-1} while no limit is indicated for DDVP. The USA Environmental Protection Agency (EPA)[40] establishes the maximum acceptable concentration for phorate in coffee as being 0.02 μg kg^{-1}, while National Secretary of Sanitary Vigilance (SNVS)[30] updated by the Brazilian Association of Nourishment Industries (ABIA) indicates the limit of phorate for coffee as being 0.05 mg kg^{-1} and for DDVP in potato 0.5 mg kg^{-1}, fruits 0.1 mg kg^{-1} and coffee 2 mg kg^{-1}. The concentration of phorate residue found in coffee is quite below the limit permitted by ANVISA, ABIA and EPA. The value of DDVP residues determinate in potatoes, guavas and coffee is also below the value permitted by ABIA.

Table 4. Analyses of organophosphorus pesticides in food samples collected in the region of Patos de Minas. The numerical indices indicate collections made at different times

Foods	Concentration of Pesticides (μg kg^{-1})		
	DDVP	Phorate	Sample number
$Potato_1$	0.15 ± 0.01	ND	8
$Potato_2$	0.10 ± 0.01	ND	9
$Potato_3$	ND	ND	6
Guava	0.26 ± 0.01	ND	5
Coffee	0.23 ± 0.06	0.02 ± 0.01	12

Conclusions

This work describes an alternative method for analyses of organophosphorus pesticides in samples of fish, with SPME-CG 100-μm PDMS fiber, which can be used in analysis of waters, fruits, potatoes and coffee.

Results indicated residues of DDVP in samples of fish (*pimelodus maculatus*) collected in the Paranaiba River. Three out of the eight samples of waters analyzed presented this pesticide. It was also present in potatoes, guava and coffee. Coffee also indicated presence of phorate. However, pesticides co-ral, di-syston, phosdrin and malathion were

detected in water. Thus, in the monitoring of nine organophosphorus pesticides, six different active groups were detected in the samples analyzed.

The method proposed in this work proved to be suitable for analysis of organophosphorus pesticides in fish, showing good precision and linearity. Limits of detection ranged from 0.005 to 1.097 μg L^{-1}, depending on the compound, except for phosdrin, whose limit of detection was 8.374 mg L^{-1}.

It is observed that the pesticide residue detected in fish was one of the organophosphorus found in samples of water collected in the Paranaiba River and its tributaries and sub tributaries, as well as in the regional samples of fruit and potato analyzed. This demonstrates that pesticides that are widely used in the agriculture and cattle breeding of Patos de Minas are being leached through rains, contaminating waters and fish of the region, as well as other foods.

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 when compared to other methods.

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