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Optimization and validation of a method based on matrix solid-phase dispersion for rganophosphorus pesticides in vegetables

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Abstract

The objective of this study was the optimization and validation of a method based on matrix solid-phase dispersion (MSPD) for the determination of organophosphorus pesticides in vegetables. This assay was carried out by capillary gas chromatography with Flame Photometric Detection (FPD) and a capillary column DB5; the recoveries were determined by fortifying six different crops (tomato, onion, green pepper, broccoli and squash) with the pesticides studied (diazinon, di-syston, methyl-parathion, malathion, parathion, ethion). The optimization of the extraction of these pesticides was achieved using MSPD with diverse extraction and purification phases, where 0.5 g of silica gel on the extraction phase and 2 g of mix of charcoal-magnesium oxide-celite (1:2:4) on the purification phase, eluted using 40 mL dichloromethane, 95% lesser than the used in the official method, were able to extract the pesticides residues. The recoveries were in the range of 61 to 108%. The regression coefficients were 0.99, except for ethion (r = 0.98) over the range between 50, 100 and 200% of maximum residue limits. The applicability of the method to detect and quantify the studied pesticides was demonstrated successfully in 32 vegetables samples obtained from two local markets. Malathion (0.08 μg/g) and parathion (0.06 μg/g), were detected in only one tomato sample, at levels lower than the maximum permit limits. The proposed analytical method could be used as an efficient, fast, cheap and ecological procedure in routine determinations of organophosphorous pesticides in vegetables.

Key words: MSPD, pesticides, vegetables, extraction methods.

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Introduction

Environmental stability is world widely in constant danger for the natural effects and the caused by the human being in its search for food satisfaction. This has produced an increase in agricultural production and the application of synthetic agrochemical like organophosphorus pesticides used on vegetables (10). Pesticides have greatly caused the present environmental unbalance and also the residues in food may cause health problems (19); besides, the international market limits exporter countries of agricultural products by the controls demanded by importers (4). This has propitiated the development of analytical, fast, efficient, cheap and safer for the environment methods, to monitor constantly the pesticides residues on food (10). The method based on matrix solid-phase dispersion (MSPD) has these characteristics and can be applied to great quantity of matrix: animal tissue (9), vegetal tissue (18), milk (8), and water (7), so it is recommendable for vegetables. The objective of this research was to optimize and validate the method of method of extraction of organophosphorus pesticides based on matrix solid-phase dispersion on vegetables of high consume in the Norwest of Mexico, a main zone of exportation nationally.

Materials and methods

Pesticides work solutions

The studied pesticides were diazinon, di-syston, methyl-parathion, malathion, parathion and ethion (Polyscience, IL., USA) selected by their use on fruit and vegetable crops (2). Pattern solutions of each pesticide were prepared, and after them work solutions at three levels of concentration 50, 100 and 200% of the maximum residue limit for tomato, established by USDA, 1991 (16) for ethion, parathion and diazinon; by the Codex Alimentarius, 2005 (3) for di-syston and mathion, and by CICOPLAFEST, 1997 (2) for methyl-parathion. Some sources were quoted to cover the analyzed pesticides, because depending on the source, the use of some pesticides is prohibited. These values were modified in the Toxic Residues Lab (TRL) (Laboratorio de Residuos tóxicos, LRT), approved by SAGARPA (Agricultural, Livestock, Rural Development, Fishing and Food Office) and accredited by EMA (Mexican entity of Accreditation), taking into account the resolution of the column for a sample size of 0.5 g used by the micro-method of extraction MSPD, compared to 25 g of sample that is established by the official method (table 1).

Gas Chromatograph

A Gas Chromatograph Varian model 3300 with Flame Photometric Detection (FPD) and a megabore column J&W Scientific DB-5 (30 m x 0.53 mm DI, 1.5 µm). The separation

Table 1. Composition and concentration of the work solutions used to analyze organophosphorus pesticides, maximum limit of residues (MLR) and minimum limit of detection (MLD).

Organophosphorus pesticides	Pattern solution mg/mL	Volume[a] mL	Work solution[b] μg/g	MLR[c] μg/g	MLD[d] μg/mL	MLD[e] μg/mL
Diazinon	169	1.479	2,0*	0.5[1]	0.005	0.005
Disyston	458	0.546	2,0*	0.5[2]	0.006	0.006
Methyl-parathion	980	0.128	1,0	1.0[3]	0.003	0.003
Malathion	990	0.253	2,0*	3.0[2]	0.008	0.008
Parathion	980	0.128	1,0	1.0[1]	0.002	0.008
Ethion	950	0.263	2,0	2.0[1,2,3]	0.003	0.002

[a] The capacity was done with dichloromethane pesticide degree at a volume of 25 mL.
[b]Work solution with adjusted MLR (*)
[c]MLR = Maximum Limit of Residues
[1]USDA, 1991
[2]Codex Alimentarius, 2005
[3]CICOPLAFEST, 1997 (Comision Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Tóxicas)
[d]MLD = Minimum Limits of Detection established by the Toxic Residues Lab from the CIAD (Laboratorio de Residuos Tóxicos del CIAD) (Centro de Investigación en Alimentación y Desarrollo) accredited by SAGARPA
[e]MLD = Minimum Limits of Detection with the method of the NOM-028-ZOO-1994.

conditions of pesticides were: Initial temperature of 190ºC by minute, a heating velocity of 3°C/min until reaching 275ºC, which was kept for 10min; the flow of the conveying gas (nitrogen) was of 30 mL/min. The temperatures of the detector and the injector were of 220°C, with an hydrogen flow of 140 mL/min and air flows of 80 and 170 mL/min; chromatographic conditions established in the Toxic Residues lab of the CIAD (Centro de Investigación en Alimentación y Desarrollo). Minimum limits of detection established in the lab of the CIAD are shown in chart 1, for this, dissolutions were made from the solutions of 50% of the maximum limit of residues of each pesticide, determining the sensibility of the team.

Selection and preparation of samples

Five vegetables of high consume in the state of Sonora were selected (17): tomato, onion, green pepper, squash and broccoli was added for its content of selenium and its anticancerigenic activity (5). At random, 10 kg of each vegetable were sampled in two malls of the locality, obtaining 32 samples as a total. Vegetables were washed, were homogenized in a Molinex blender model 648, they were packed in bags in portions of 200 g, they were labeled and storaged at 20ºC until analysis. To dry the samples was not necessary because according to the methodology of the Official Mexican Norm (Norma Oficial Mexicana) NOM-004-ZOO-1994, the process is done on a humid base including controls (11).

Data quality

During the optimization and validation of the method, a negative control (pesticides free tissue) was added, a positive control (Tissue with work solution at 100% of the maximum limit of residues).

Optimization

Purification and extraction method

Different solid phases of extraction and purification were used as silica (60 ú pore size, Baxter, USA), alumina, octadecylsilane (C-18) and a mixture of absorbents, which contains activated charcoal, magnesium oxide, and celite in portions 1:2:4, respectively, prepared according to the NOM-028-ZOO, (12). They were homogenized in a mortar 0.5 g of the extraction phase with 0.5 g of every added vegetable with 100 ìL of the work solution of pesticides, corresponding to the 100% maximum limit of residues (Homogeneous mixture). This homogeneous mixture was packed in glass or plastic syringes of 5 or 10 mL respectively, previously packed with the purification phase. The elution was proved at different volumes (15, 20, 40, 60 mL) of hexane or dichloromethane pesticide degree, to determine the volume and the eluding dissolving which reached highest recuperation percentages. The elution was obtained in a test tube of 50 mL and was evaporated until dried in an air evaporator (Organomation Associates, Inc. Berlin, MA. 01503, USA) at 40°C.

Chromatographic analysis of pesticides

The evaporated extract was reconstituted with 100 µL of dichloromethane and 2 µL were injected in the chromatograph of gas. The percentages of recuperation were obtained through the external standard method proposed by Hammarstand, 1976 (7), which compares the time of retention and the obtained areas of the resultant chromatogram of the work solution, with the times and the areas of each obtained pesticide in the positive solution with known concentrations of analytes of interest. The analysis of problem samples was made by extraction triplication and an injection of every extraction.

Extraction method validation

Equipment validation. Chromatographic conditions of the equipment were established and the times of retention on analytes, also the limits of detection to each standard pesticide (figure 1).

Phase I. Linearity of the system

During this phase, the linearity of the method was evaluated, which is demonstrated through calibration curves, based on the reference pattern.

Preparation of pesticides' work solutions. There were prepared work solutions of every analyte at three levels of concentration 50, 100 and 200% of the maximum limit of residues. This lasted three days and every triplicate of determination, was

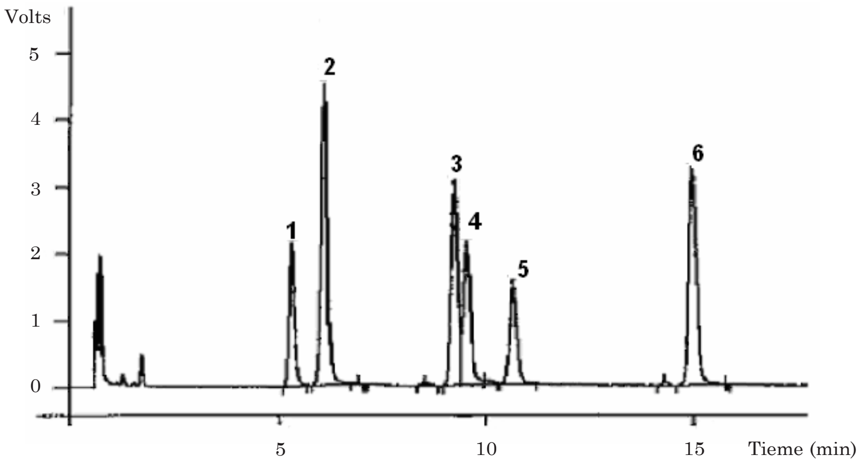

Figure 1. Regular Chromatogram of work solution at 100% of MLR of organophosphorus pesticides. (1) Diazinon, time of retention (TR) = 5.321 min; (2) Disyston, TR = 6.110 min; (3) Methyl-parathion, TR = 9.219 min; (4) Malathion TR = 9.525 min; (5) Parathion TR = 10.632 min; (6) Ethion TR = 14.991 min.

injected by triplicate in the chromatograph. Graphics of concentration were obtained from the chromatograms, area and the coefficient of correlation.

Phase II. Precision of the method (Repeatability)

a. Considering that the method of extraction was similar for the six vegetables of study, a sample of tomato was taken as positive control. This sample was previously analyzed and was pesticides free, so it was taken to determine the precision of the method.

b. The positive control was added to pesticides' work solutions at levels of 50, 100 and 200% of the maximum limit of residues. Analyses were done during three days and every determination was made by triplicate of extraction with an injection in the chromatograph of each extraction. With the analysis results were calculated the percentages of recuperation and variation coefficients for every analyte and the established parameters by USDA, 1991 (15) were compared.

Phase III. Accuracy of the method

During this phase, the accuracy of the method was evaluated through verification simples. The coordinator of quality of TRL prepared these samples, establishing the levels to add the analytes. Concentrations and percentages of recuperation were obtained, and then compare the obtained concentrations with the theoretic established concentrations.

Statistical analysis

Obtained data during optimization and validation of the method were analyzed with descriptive statistics considering the average, standard deviation, variation coefficient, correlation coefficient of calibration curves of each pesticide and an analysis of variance with test Z (P<0.05) was done in the linearity of the system and the precision (repeatability) of the method.

Results and discussion

Optimization

During optimization of the method MSPD, the following parameters were used: Extraction Phase, Purification Phase, Chromatographic Column Type, Volumes and elution solvents (table 2). This chart shows the most relevant modifications results of parameters of extraction, where the major recuperations were done with the following parameters: 0.5 g of silica for the extraction, 0.5 g of the mixture of absorbents (activated charcoal, magnesium oxide and celite 1:2:4) for purification, a glass syringe of 5 mL as chromatographic column and 40 mL of dichloromethane as elution. These extraction conditions were selected considering that the obtained extract showed less interference and color. The optimization was carried put with tomato and was subsequently proved with the rest of the vegetables (table 3), where high results of recuperation were reached.

The proposed MSPD method in this research, promoted a considerable diminishing in the use of solvents, obtaining a reduction of 95% in the use of dichloromethane according to the official method (13), whereas others methods diminished only from 31% (10) to 81% (12), excepting the method proposed by Anastassiades and Lehotay (1), that had a diminish of 98%. However, this method has an increment of the operation cost that affects the analysis cost, since it needs the use of refrigerated centrifuges, stirring rods, evaporators, among others, which is not required for the proposed MSPD method. The obtained percentages of recuperation were between the ranges of 61 to 108%, with variation coefficients minors to 8.7%, which fulfill the parameters established by the USDA, 1991 (15), assuring that the MSPD method is proper for its application (table 3).

Validation of the method

Phase I. Linarity of the system

The obtained correlation coefficients were of 0.99 for all pesticides except for the ethion, which was of 0.98. This indicates that the system preserves the expected linearity. Equally, the variation coefficients fulfill the range of established specifications. The variance analysis showed that there were not significant differences (P<0.05) between every day repetitions, during the three days, for every concentration and pesticide, which shows that the linearity was kept in the analysis of the system.

Phase II. Precision of the method (Repeatability)

The precision parameter was

Table 2. Optimization of parameters of the extraction methods and purification of residues of pesticides in vegetables based on matrix solid-phase dispersion.

Extration phase g	Purification phase g	Elution solvent mL	Sample g	Chromatographic column DI/ L mm	Percentage of recuperation %
		Hexane			
0.2 MA	0.5 A	15	0.1	5/110	40-82
0.2 MA	0.5 A	30	0.5	5/110	55-93
0.1 A	0.1 S	40	0.1	5/110	45-79
		Dichloromethane			
0.5 C-18	0.5 S	30	0.5	15/75	51-86
0.5 MA	0.5 C-18	30	0.5	15/75	45-90
0.5 MA	0.5 MA	40	0.5	20/95	53- 93
0.5 S	0.5 MA	20	0.5	15/75	49-89
0.5 S	0.5 MA	30	0.5	15/75	58-101
0.5 S	0.5 MA	40	0.5	15/75	65-110

AM = Absorbent Mixture (activated charcoal-magnesium oxide-celite 1:2:4)
C-18 = Octadecylsilane
S = Silica
A = Alumina

Table 3. Recuperation percentages of variation of organophosphorus pesticides in vegetables through the MSPD optimized method.

Pesticides	Tomato 1		Tomato 2		Onion		Broccoli		Squash		Green pepper	
	%	CV	%	CV	%	CV	%	CV	%	CV	%	CV
Diazinon	94	3.8	98	7.5	99	3.1	98	8.7	96	6.9	95	4.6
Disyston	64	5.9	61	2.8	61	1.6	61	1.9	62	4.3	62	2.5
Methyl-parathion	97	2.1	100	4.4	92	3.8	98	4.1	94	4.3	105	0.6
Malathion	102	4.4	104	7.8	108	2.5	105	3.6	104	3.1	103	1.5
Parathion	101	7.0	97	2.6	101	5.6	98	3.3	100	3.1	103	1.5
Ethion	94	0.6	98	6.0	99	7.6	92	3.6	94	2.1	102	8.0

The values of the recuperation percentages and variation coefficient correspond to a triplicate average of extraction and a determination through extraction.

evaluated obtaining the percentages of recuperation and variation coefficients. The obtained recuperation percentages at the three levels of concentration of used pesticides, were between the ranges of 65 to 102% with variation coefficients minors to 12.4% (table 4), which shows that the proposed method fulfill the specifications and can be applied safely and efficiently. The variance analysis indicated that there were not significant differences ($P<0.05$). The method kept its precision.

Phase III. Accuracy of the method

Table 5 shows the accuracy results. In this phase, four samples were evaluated by duplicated, adding known concentrations of pesticides patterns. Obtained concentrations for every analyte were adequate and were between ranges of 89 to 111%. These values fulfill the parameters established by the USDA, 1991 (15). The method showed the necessary accuracy to consider it as an adequate method for its application.

Analysis of vegetables

Once the method was validated, the analysis of the vegetables from the local commerce, took place. From all the analized samples, only a tomato sample presented 0.06 µg/g of malathion and 0.08 µg/g of parathion, values under the maximum limits of residues (table 1) and similar to the ones found by Peres *et al*., 2002 (14) therefore, its consume do not represent any harm for health.

Table 4. Percentages of recuperation of organophosphorus pesticides in simples of fortified tomatoes at 50, 100 and 200% of the maximum limit permitted (Phase II. Repeatability of the Method).

Pesticides	Recuperation			CV[a] %			Especification[b] %	
	50	100	200	50	100	200	Recuperation %	CV[a] %
Diazinon	92	89	89	10.7	12.4	12.1	60-100	≤20
Di-syston	65	68	65	7.3	7.4	7.1	60-110	≤20
Methyl-parathion	96	99	92	8.6	5.6	10.1	70-110	≤20
Malathion	97	98	93	9.5	6.7	8.0	70-110	≤20
Parathion	101	102	95	7.9	5.2	7.6	65-110	≤20
Ethion	92	98	100	8.1	7.9	6.6	65-110	≤20

[a]Variation coefficients
[b]USDA, 1991

Table 5. Obtained recuperations in tomato samples contaminated with different concentrations of organophosphorus pesticides (Phase III. Accuracy of the Method).

Pesticides	Theorycal concentration of the compound µg/g	Obtained concentration by the analyst ± DE µg/g	CV %	Obtained recuperation percentage by the analyst %	Percentage of adequation[a] %	Accepted recuperation percentage and adequation %
Metil-Paratión[1]	1.00	1.03±0.034	3.30	95	103	70-.110
Etión	1.98	1.97±0.1	5.11	92	99	60-110
Dysistón[2]	4.02	4.08±0.11	2.69	70	102	65-110
Paratión	1.99	2.21±0.055	2.49	90	111	65-110
Diazinón[3]	1.00	0.89±0.01	1.12	80	89	60-110
Malatión	0.99	1.046±0.15	0.11	99	105	70-110
NA[4]	ND	ND	NN	NN	NN	NN

[a]Relation between theoretical concentration and the obtained concentration, expressed in percentages.
CV = Variation Coefficient DE = Standard Deviation, NA = No added, ND = No detected y NN = Not necessary.
[1]Added Sample at 100% MLR with pesticides (Methyl-parathion and Ethion),
[2]Added Sample at 200% MLR with pesticides (Dy-siston y Parathion),
[3]Added Sample at 50% LMR with pesticides (Diazinon y Malathion)
[4]Sample without pesticides added.

Conclusions

The Method based on Matrix Solid-Phase Dispersion proposed in this research, it uses silica as extraction base and a mixture of activated charcoal, magnesium oxide and celite for the purification phase, was optimized and validated fulfilling the parameters of quality specified by USDA, 1991 (15). This method, promoting a decrease in the use of solvents helps with the safety of environment, and because is simple and cheap, can be applied as a routine technique of analysis to monitor vegetables of national consume and exportation.

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