DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN FRUITS AND VEGETABLES BY MATRIX SOLID-PHASE DISPERSION METHOD

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Abstract: Multiresidue matrix solid-phase dispersion (MSPD) technique with capillary gas chromatographic detection for determination and quantification of ten commonly used organophosphorus pesticides (chlorpyrifos, chlorpyrifos-methyl, diazinon, dimethoate, fenitrothion, formothion, malathion, parathion, phosalone, pirimiphos-methyl) in fruits and vegetables is described. The method gave high recoveries for all spiked samples in 80–120% range. The limits of detection were from 0.01 to 0.1 mg/kg for the compounds studied and were much lower than maximum residue levels (MRLs) allowed. The procedure was applied to the determination of ten organophosphorus pesticides in samples collected during the routine crop monitoring.

Key words: organophosphorus pesticide, residue, solid-phase, matrix, gas chromatography

INTRODUCTION

Organophosphorus compounds are the most widely used insecticides nowadays. They are commonly used in agriculture on a variety of crops to control pests causing considerable damages to plants. The use of pesticides provides an unquestionable benefit for agricultural production. Despite of the fact that the organophosphorus pesticides are often present at trace level, they have an influence on the environment as well as on the human health due to their toxicity. Therefore, monitoring of pesticide residue levels in fruits and vegetables is necessary to protect consumers' health.

Most analytical methods used for the determination of pesticide residues in plant samples use solvent extraction followed by liquid-liquid partitioning, clean-up procedures and gas chromatography detection (Luke et al. 1975; Ambrus et al. 1981). This usually takes hours and requires large volumes of organic solvents. In the recent years the main attention has been paid to simplification of the

sample preparation and elimination of toxic organic solvents from analytical proce-
dures. Matrix solid-phase dispersion (MSPD) method can help to reduce consider-
ably size of the sample and usage of solvents. It was introduced by Barker et al.
(1989; 1993) for isolation of drug residues from biological samples. For pesticide
analysis it has been used in samples of milk (Di Muccio et al. 1996), fish (Ling and
Huang 1995), beef fat, crayfish (Barker 2000) and to analyze multiresidues in fruits
and vegetables (Fernandez et al. 2000; Torres et al. 1996; Viana et al. 1996). It com-
bines several analytical techniques in one step and is relatively easy to perform. It is
designed to disperse a small amount of sample on a solid-phase that is being used
as a column packing material from which analytes can be eluted with a small vol-
ume of solvent. Our success with the MSPD method for other pesticide/matrix
combinations (Morzycka 2002) led us to evaluate the MSPD approach for determi-
nation of organophosphorus insecticides.

The main purpose of the present work was to demonstrate that the MSPD
method can be useful alternative for the conventional techniques used for the sepa-
ration of pesticides from fruits and vegetables.

MATERIALS AND METHODS

Chemicals. Analytical reference standards of 95–99% purity were supplied by Dr.
Ehrenstorfer Laboratory (Germany). Standard stock solutions of various concen-
trations (1–5 mg/ml) in acetone were prepared and stored at 4°C. Analytical grade
solvents: acetone, n-hexane, methylene chloride and ethyl ether were distilled, if
necessary. The solid-phase materials used for this study were Florisil PR (Floridon
Company, USA) and Silicagel 60 for column chromatography (63–200 mesh,
Merck). Both sorbents were activated by oven heating at 500°C for 4 h, allowed to
cool and stored in stoppered bottles. Anhydrous sodium sulfate p.a. used for drying
was heated at 500°C for 4 h. Deionized water purified with a Milli-Q water purifica-
tion system (Millipore Corporation) was used.

Sample preparation. A representative portion of the sample (200±1 g) was
chopped into small pieces in order to obtain a material easy to mix. The pulps of
fruits or vegetables were prepared using a high-speed blender. In some cases, dis-
tilled water was added for better blending (Tab. 1). The portion of the pulp repre-
senting 5±0.1 g of sample was placed into a mortar and an appropriate weight of
silica was added. To obtain powdered and homogenous mixture of sample dis-

Table 1. Amount of water and silica required for preparing samples for MSPD extraction

Commodity	Water added* [ml]	Weight of pulp [g]	Weight of silica [g]
cherry	–	5	8
apple	50	6.25	10
lettuce	100	7.5	12
carrot	100	7.5	12
black currant	100	7.5	12
cabbage	100	7.5	12
red beet	100	7.5	12

* For 200 g of sample

persed on the solid phase the pulp was mechanically blended with a sorbent using a glass pestle. This mixture was placed into a glass column (200 x 15 mm i.d) containing additional 2 g of silica and 3 mm layer of anhydrous sodium sulfate at the bottom. For cabbage and cauliflower samples there was used the mixture: silica + activated carbon (1.5 g + 0.5 g) instead of 2 g of silica. Pesticide residues were eluted with a 60 ml of mixture of solvents (dichloromethane-acetone, 9 : 1 v/v). Elution was performed by gravity flow. The eluates were evaporated to dryness using a vacuum evaporator and dissolved in 5 ml of *n*-hexane.

Recovery studies. Recoveries of the pesticides were determined by two methods: MSPD and classical extraction with acetone (Luke et al. 1975). Untreated samples were spiked with a known quantity of pesticide standard solutions in three levels, depending on the detection sensitivity of each compound. The pesticide concentrations in spiked samples were 0.05–0.5 mg/kg, in all cases below the maximum residue levels (MRLs) recommended for the analysis of pesticides. Recoveries were calculated from a five-point calibration curve in the range of 0.01–1.0 mg/kg. Recoveries of pesticides were tested in three replicates to calculate relative standard deviations (RSD%). Calibration curves for all standards were constructed in the linear concentration range. The correlation coefficients were above 0.9990.

Analysis of organophosphorus pesticide residues. Pesticides residues were determined by gas chromatography using a HP 5890 instrument and HP-5 (5% PH ME Siloxane, 10 m, 0.53 mm, film thickness – 1.5 mm) capillary column. Operation conditions: temperature program, 160°C for 1 min, increasing 10°C/min to 270°C and holding 2 min. Injection port temperature 210°C, detection was accomplished using a nitrogen-phosphorus detector at 240°C. Recoveries for all compounds were determined by comparison of the data obtained from extracted samples with the data obtained by direct analysis of standards.

RESULTS AND DISCUSSION

In the present study the sample was dispersed over non-modified silica. This is the major difference of the method from the typical MSPD methods in which the dispersion step is usually made on lipophilic octadecylsilica (C_{18}) allowing for easy removal of non-polar fatty materials from samples like milk or meat. In the case of pesticide analysis in fruits and vegetables this function of the solid support is less important.

The ten organophosphorus pesticides selected for this study are among the most frequently plant protection products used in crops. Investigations were performed for various pesticide/matrix combinations (Tab. 2). Two different extraction procedures: MSPD and liquid-liquid with acetone were compared with each other with regard to their suitability for isolation of the pesticides from fruits and vegetables. The recovery data of ten pesticides are listed in tables 3 and 4. The results presented here were calculated from GC analysis with NP detector. In general, there is no significant difference between the recoveries of the tested MSPD and the Luke's methods. For all compounds acceptable recoveries were obtained for each commodity in 80–120% range. Reproducibility calculated as relative standard deviation (RSD%) for various pesticides amounted from 1% to 10% for both methods. The limit of de-

Table 2. Pesticides and matrices included in the studies

Pesticide	Cherry	Apple	Black currant	Carrot	Cabbage	Lettuce	Red beet
chlorpyrifos		x			x		x
chlopyrifos-methyl		x			x		x
diazinon	x	x		x	x	x	x
dimethoate					x		x
fenitrothion	x	x	x	x	x		x
formotion					x		
malathion			x	x	x		
parathion		x					
phosalone	x	x	x	x	x		
pirimiphos-methyl		x			x	x	

tection (LOD), defined for convenience as the quantity of analyte which generates a response 3 times greater than the noise level of the detection system, was in the range 0.01–0.08 mg/kg. The methods are equally efficient and allow determining of a broad range of compounds. However, the MSPD method offers an important saving of time, reduces the sample size, requires less solvent for efficient extraction of analyzed compounds in comparison with the classical multiresidue methods. The consumed solvent's volumes were 60 ml with the MSPD method, whereas 510 ml with the classical method. The extracts obtained were clean and could be successfully analyzed using capillary gas chromatography with selective detectors such NP or EC. No further purification step was necessary prior to analysis except for the cabbage and the cauliflower samples which required additional clean-up due to the presence of interfering peaks from the matrix. Using clean-up adsorbent at the bot-

Table 3. Recovery data and standard deviations obtained by the MSPD method for apple and cabbage samples

Pesticide	Level of spike [mg/kg]	Mean recovery [%]	RSD [%]	LOD [mg/kg]	MRL [mg/kg]	Mode of detection
Apples:						
chlorpyrifos	0.08	93.6	2.1	0.03	0.5	NPD/ECD
chlorpyrifos-methyl	0.1	100.4	1.8	0.03	0.5	NPD/ECD
diazinon	0.1	84.5	2.6	0.03	0.3	NPD/ECD
fenitrothion	0.1	103.0	3.9	0.02	0.5	NPD/ECD
parathion	0.1	99.3	4.9	0.04	0.1	NPD
phosalone	0.2	91.5	5.3	0.05	2.0	NPD/ECD
Cabbage:						
chlorpyrifos	0.06	98.6	2.0	0.03	0.1	NPD/ECD
chlorpyrifos-methyl	0.08	87.3	1.2	0.05	0.1	NPD/ECD
diazinon	0.05	92.2	2.8	0.02	0.3	NPD
dimethoate	0.2	120.0	8.8	0.08	0.5	NPD
fenitrothion	0.1	94.0	5.7	0.05	0.5	NPD/ECD
formothion	0.08	89.1	4.5	0.03	0.1	NPD
malathion	0.1	88.8	5.4	0.03	0.5	NPD
phosalone	0.2	83.7	2.9	0.05	1.0	NPD/ECD
pirimiphos-methyl	0.1	89.9	3.2	0.02	0.2	NPD

Table 4. Recovery data and standard deviations obtained by the Luke's method for apple and cabbage samples

Pesticide	Level of spike [mg/kg]	Mean recovery [%]	RSD [%]	LOD [mg/kg]	MRL [mg/kg]
Apples:					
chlorpyrifos	0.06	95.0	1.2	0.05	0.5
chlorpyrifos-methyl	0.2	90.2	1.0	0.05	0.5
diazinon	0.1	89.0	1.8	0.04	0.3
fenitrothion	0.2	91.7	1.3	0.05	0.5
parathion	0.06	102.2	9.3	0.03	0.1
phosalone	0.5	100.5	3.6	0.05	2.0
Cabbage:					
chlorpyrifos	0.06	92.1	2.1	0.03	0.1
chlorpyrifos-methyl	0.08	102.2	2.0	0.05	0.1
diazinon	0.1	88.2	3.2	0.04	0.3
dimethoate	0.3	118.1	4.1	0.1	0.5
fenitrothion	0.2	93.9	3.8	0.05	0.5
formothion	0.08	112.3	2.0	0.04	0.1
malathion	0.2	97.7	1.7	0.04	0.5
phosalone	0.3	92.6	5.2	0.05	1.0
pirimiphos-methyl	0.1	88.3	2.9	0.02	0.2

tom of the column minimized such interferences. The purification of the extract was tested with several adsorbents like neutral alumina, silica, Florisil, activated carbon or their combinations. Using silica as the disperse phase and silica with activated carbon as the cleaning adsorbent gave the best recoveries. For some compounds the recoveries were also calculated from GC analysis with EC detector but no significant differences were observed. In some cases the ECD chromatograms showed more matrix peak signals than NPD. The described procedure was applied for determination of organophosphorus pesticides in fruit and vegetable samples collected during monitoring program, supporting the results obtained by the classical method.

In conclusion the MSPD extraction is very simple, rapid and cost-effective. The use of this method is beneficial when compared with the classical solvent extraction due to elimination of the partition step often causing emulsion problems and a number of manual operations that affect the results of analysis. The method is useful for organophosphorus pesticide monitoring. The screening of organophosphorus pesticides can be carried out reliably with the majority of commodities by capillary GC.

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POLISH SUMMARY

OZNACZANIE POZOSTAŁOŚCI PESTYCYDÓW FOSFOROORGANICZNYCH W OWOCACH I WARZYWACH METODĄ ROZPROSZENIA MATRYCY W FAZIE STAŁEJ

Celem badań było sprawdzenie możliwości zastosowania metody rozproszenia matrycy w fazie stałej (MSPD) do oznaczania pozostałości najczęściej stosowanych związków fosforoorganicznych w owocach i warzywach. Porównano wyniki oznaczeń dziesięciu najczęściej stosowanych insektycydów z tej grupy związków uzyskane dwiema metodami: MSPD i klasyczną z wykorzystaniem ekstrakcji rozpuszczalnikiem organicznym. Obie metody okazały się równie skuteczne, a ich parametry (wartości odzysku, odchylenie standardowe, granice oznaczalności) porównywalne. Metoda MSPD jest jednak prostsza, mniej czasochłonna, a przede wszystkim bardziej ekonomiczna od metody klasycznej. Ograniczenie zużycia rozpuszczalników organicznych jest ważne ze względu na ich negatywny wpływ na zdrowie osób pracujących w laboratorium i środowisko.

Metodę MSPD zastosowano w analizie pozostałości związków fosforoorganicznych w monitoringowych badaniach próbek owoców i warzyw.