Determination of Organophosphate Pesticides in Vegetable Farm Drained Water Using Solid Phase Extraction Followed by High Performance Liquid Chromatography

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Organophosphorus pesticides in water from four vegetable farms in Patumtanee Province were analyzed by solid-phase extraction (SPE) and reverse-phase high performance liquid chromatography (HPLC). SPE experiments were performed under conditions of an ODS phase, a sorbent mass of 100 mg, solution volume of 100 ml, elution solvent of 60/40 acetonitrile/water, elution volume of 5 ml and pressure of 17 in. Hg. Extraction method recovery was confirmed by spiking standard pesticides at 1 ppm. Quantification by an external standard method implied a 90% average recovery of standard profenofos. Profenofos was detected in 27 water samples from three vegetable farms. The detected concentration range was 0.11 to 1.11 ppm with a mean of 0.44 ± 0.07 ppm. The chemical structures of pesticides were identified by mass spectra.

Key words: Pesticide, organophosphate, solid phase extraction.

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การตรวจหาสารกำจัดศัตรูพืชออร์แกโนฟอสเฟตในน้ำจากไร่ผักโดยการสกัดด้วยวัฏภาคของแข็งตามด้วยเทคนิคไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโทกราฟี

ปราณี เทศกะทึก วนิดา จีนศาสตร์ และ ปีเตอร์ อาร์โนลด์ (2544)
วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **26(1)**

ตรวจพบสารกำจัดศัตรูพืชออร์แกโนฟอสฟอรัสในน้ำจากไร่ผักสี่แห่งในจังหวัดปทุมธานี การวิเคราะห์ตัวอย่างน้ำทำโดยใช้การสกัดด้วยวัฏภาคของแข็งและไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโทกราฟี สภาวะการสกัดใช้สารดูดซับออกตะเดคซิลเคลือบบนซิลิกา 100 มก. ปริมาตรน้ำ 100 มล. ตัวชะใช้ 60/40 อะซีโตไนไตรท์ / น้ำ ปริมาตรของตัวชะ 5 มล. และความดัน 17 นิ้ว ปรอท การสกัดทำให้ได้ค่าเปอร์เซ็นต์คืนกลับของสารโปรเฟนโนฟอส เฉลี่ย 90% โดยใช้สารมาตรฐานเริ่มต้น 1 พีพีเอ็ม ผ่านขั้นตอนการสกัดและการวิเคราะห์ตามสภาวะที่กล่าวข้างต้น คำนวณหาความเข้มข้นจากกราฟที่พล็อต ระหว่างความเข้มข้นกับพื้นที่ใต้พีคของ HPLC ตัวอย่างน้ำทิ้งในร่องผัก 27 ตัวอย่างจากไร่ผักสามแห่ง ตรวจพบโปรเฟนโนฟอสมีความเข้มข้นอยู่ในช่วง 0.11 ถึง 1.11 พีพีเอ็ม ค่าเฉลี่ย 0.44 ± 0.07 พีพีเอ็ม สูตรโครงสร้างทางเคมีของสารออร์แกโนฟอสเฟต ตรวจสอบจาก ข้อมูลวิเคราะห์ด้วยแมสสเปกโทรสโคปี

คำสำคัญ สารกำจัดศัตรูพืช ออร์แกโนฟอสเฟต การสกัดด้วยวัฏภาคของแข็ง

INTRODUCTION

The use of organophosphorus insecticides has been increasing since the withdrawal of organochlorine insecticides due to their toxicity, persistence and bio-accumulation in the environment. On the contrary, most organophosphate compounds are not so persistent and only stay active for a few hours to several months. However, these insecticides break down fairly rapidly, generating by-products that are toxic to aquatic microorganisms, fish, birds, humans and other mammals.(1) Because of their non-persistent activity, farmers have to apply insecticides at regular intervals to ensure their effective insect control. This results in continuous accumulation of chemicals in the environment.

The aquatic systems could take up pesticides from several sources such as from direct application, spray drift, aerial spraying, washing from the atmosphere by precipitation, erosion and run-off from agricultural land, discharge of effluent from factories and sewage. In some cases, the main source of water pollution is the agricultural run-off with pesticides that contaminate the water system and the land in the nearby areas.

In Thailand, the organophosphates have been widely used and imported in high quantities. The Agricultural Regulatory Division reported that more than 5,000 tons of organophosphates were imported per year, and costing more than 500 millions baht.(2) Many types of organophosphorus compounds were used in agriculture such as malathion, methyl parathion, chlorpyrefos, profenofos, etc. Pesticides applied during crop cycles could contaminate surface water, ground water and aquatic organisms. Recently, there have been concerns of the risk and danger a rising from pesticide usage in large areas. Several methods have been developed to monitor pesticide contamination. Analysis of pesticides in water samples normally uses either liquid-liquid extraction or solid-phase extraction followed by chromato-graphy techniques.(3) Gas chromatography and liquid chromatography coupled with mass spectrometry were used to identify unknown pesticides in water.(4-6) Solid-phase extraction has been accepted as a concentration step for water samples prior to analysis.(5)

In this study, a rapid and simple analysis method was developed using coupling solid-phase extraction with reverse phase high performance liquid chromatography (HPLC) to determine the organophosphate pesticides in water from agricultural drainage. The sampling sites were vegetable farms in Patumtanee province where large farming areas are close to the irrigation water channels. This is a pilot study for pesticide monitoring in aquatic environments. The information on usage of pesticides was very limited during our study.

MATERIALS AND METHODS

Materials

HPLC grade solvents and C18 SPE cartridges were from J. T. Baker, U.S.A. Water was doubly distilled and filtered through Millipore membranes of pore size 0.45 μm. Standard organophosphates of purity 99%, malathion, methyl parathion and chlorpyrefos, were purchased from Soekawa Chemical Co., Ltd., Japan. Profenofos, purity 99.4%, was donated by Ciba-Geigy (Thailand). Stock standard solutions were prepared in acetonitrile and stored at -20^{0}C. The Visiprep. SPE Vacuum Manifold 5-7030 Supelco was used in solid phase extraction.

Standard solutions and calibration

Stock standard profenofos (1000 μg/ml) was prepared in acetonitrile and stored at –20^{0}C. The working standard solutions were 5.0, 10.0, 20.0, 30.0 and 40.0 μg/ml, respectively. A blank test solution was 100 ml of distilled water. The external standard calibration was obtained by plotting the concentration against the peak area of chromatograms. Figure 1 shows a standard calibration curve of profenofos with $R^2 = 0.9749$. The extracted water samples were quantified from this calibration curve using the following equation:

$$\text{Conc. of profenofos residue in water} = \frac{\text{Conc. read from standard curve} \times \text{eluent volume}}{\text{Sample volume}}$$

Figure 1. Standard calibration curve of profenofos (R^2 = 0.9749).

Percentage recovery test

The solid-phase extraction experiments used Sep-Pak C_{18} (Octadecyl bonded silica) cartridges. The extracted parameters, quantity of sorbent, type and volume of solvent, and vacuum pressure, were determined. The extracted conditions giving a high % recovery were tested with a standard pesticide concentration of 1 ppm and quantified from a standard calibration curve in Figure 1. The protocol of varied parameters and the results of related percentage recovery of profenofos are summarized in Table 1. From this percentage recovery experiment, we found the extracted conditions for water sample extract. All parameters tested we chose the highest percentage recovery except the elution volume. We used 5 ml of 60% acetonitrile/water, yielding 88.8% recovery, instead of 7 ml, yielding 89.3% recovery, because we would like to minimize the elution volume used.

Water Sampling

Agricultural drained water samples were collected from four vegetable farms in Bangbuatong district, Patumtanee province (a northern suburb of Bangkok). Figure 2 is a drawing of the four sampling sites. All sites were about 500 meters from the main road. The main vegetable grown in these farms was Chinese broccoli. Thirty-six water samples were collected from the middle of furrows at 30-50 cm depths within 30-day intervals during the farming periods for three months. Each two litres of water was collected and kept in separate dark glass bottles and cooled in ice boxes during the transportation. The preconcentration samples were prepared within one day after sampling.

Table 1. Protocal of SPE parameters and percentage recovery of profenofos at 1 ppm. Experiments were performed in triplicate.*

Exp.No.	Sorbent mass (mg)	Sample volume (ml)	Elution solvent (ACN/H_2O)	Eluent volume (ml)	Pressure (in. Hg)	% recovery	% RSD
I. Sorbent Mass	**100**	**100**	50/50	5	10	**100.22**	**±2.65**
	200	↓	↓	↓	↓	81.35	±2.03
	300	↓	↓	↓	↓	33.85	±3.54
	400	↓	↓	↓	↓	13.47	±3.48
II. Sample volume	**100**	**100**	↓	↓	↓	**99.57**	**±0.71**
	↓	200	↓	↓	↓	99.91	±0.66
	↓	300	↓	↓	↓	84.87	±0.49
	↓	400	↓	↓	↓	90.97	±0.64
III. Elution solvent	↓	100	40/60	↓	↓	9.65	±0.21
	↓	↓	50/50	↓	↓	61.99	±1.31
	↓	↓	**60/40**	↓	↓	**99.89**	**±1.92**
	↓	↓	70/30	↓	↓	68.76	±1.49
	↓	↓	80/20	↓	↓	61.87	±0.24
IV. Eluent volume	↓	↓	60/40	3	↓	47.96	±1.27
	↓	↓	↓	4	↓	64.71	±0.69
	↓	↓	↓	**5**	↓	**88.84**	**±0.43**
	↓	↓	↓	6	↓	87.58	±0.49
	↓	↓	↓	7	↓	89.32	±0.70
V. Pressure	↓	↓	↓	5	10.0	84.24	±1.07
	↓	↓	↓	↓	12.0	85.15	±0.21
	↓	↓	↓	↓	15.0	88.78	±1.02
	↓	↓	↓	↓	**17.0**	**90.78**	**± 0.75**
	↓	↓	↓	↓	20.0	85.91	±0.59

*Selected conditions are in bold type.

Figure 2. Sampling sites (I-IV) at vegetable farms in Bangbuatong District, Patumtanee Province.

Organophosphates determination

A water sample of 100 ml was aspirated through a 100 mg preactivated sep-pak C_{18} cartridge at pressure 17 in. Hg. Trapped pesticides were eluted with 5 ml of 60% acetonitrile in water and then dried under nitrogen gas flow. These concentrated samples were dissolved in 100 µl of 60% acetonitrile. Extracted samples 20 µl were injected to HPLC LDC4100 equipped with UV detector operated at µ 220 nm; flow rate 1 ml/min; detector sensitivity, 0.05 AUFS; column, ODS (Octadecyl bond silica), 12.5 cm × 4 mm ID; injection interval, 15 min. The chemical structures of separated samples were identified by a GC/MS spectrometer JMS-DX 300 (JEOL). The Electron Ionization (EI) mass spectra were measured at 70 eV, 150^0 C.

RESULTS AND DISCUSSION

In our study, we selected four widely used pesticides, namely methyl parathion, chlorpyrifos, malathion and profenofos, as standard pesticides in order to compare the retention time with unknown water extracted. The HPLC graph of standard pesticides is shown in Figure 3. Examples of HPLC traces of water extracted from Farms I and II are shown in Figure 4. Comparing with the standard graph, these two chromatograms had main peaks at retention times 7.44 and 7.45 min, which were related to the retention time of standard profenofos at 7.52 min in Figure 3.

The chemical structure of the extracted compounds was identified by mass spectra. Figure 5 shows MS spectral data of components at retention time 7.5 min. The interpretation of mass spectra is shown in Table 2. The mass spectra had molecular ion clusters at m/z = 372, 374 and 376 consistent with compounds containing Cl and Br and fragment ions at m/z = 206, 208 and 210 and other Br containing ions. The halide-containing ions were in the correct relative abundance ratio.(7) The inset spectrum in Figure 5 is a spectrum from the NIST Library, which was run by matching the molecular formula of profenofos. The spectrum from the library search program was well correlated with our MS spectra. However, after comparison with the standard mass spectrum, we found two impurity ions at m/z 149 and 177. These two peaks indicated the presence of diethyl phthalate.(7) Phthalates are some impurities, usually found in mass spectra,(8) which are the common components of plasticizers contained in tubing, cap liners, and cascade and column packing.

Profenofos was detected in 27 water samples from three vegetable farms with the concentration range from 0.11 to 1.11 ppm and the average concentration of 0.44 ± 0.07 ppm. Table 3 shows the results for profenofos found in sampling sites I, II, III and IV. The first sampling from Farm I had profenofos at an of average concentration 0.75 ± 0.06 ppm, with vegetables fully grown and ready for the harvest. In the second and third samplings, profenofos concentrations were 0.38 ± 0.05 and 0.13 ± 0.05 ppm, the which were at the harvest period and the end of crop cycle, respectively. In sampling site II, the results were similar to those for site I and also the crop cycle was matched in the same period but the concentration of profenofos was lower than in Farm I. It is possible that this farm used a lower pesticide quantity than site I. However, profenofos in sampling site IV was the highest through out the crop cycle period, indicating the heavy used of pesticides. Although the profenofos residue decreased in the 10th and 20th intervals, pesticide accumulation could potentially occur if profenofos was continually applied. In farm III, our comparison site, profenofos was not detected.

Figure 3. HPLC chromatogram of a standard solution containing 100 ng of each pesticide:

Figure 4. HPLC chromatogram of water sample extracts from vegetable farms. (a) Farm I (b) Farm II.

Figure 5. The mass spectrum of the main peak eluted at 7.5 min. See text for details.

Table 2. Interpretation of profenofos mass specturm.

m/z	characteristic ions	relative abundances
376	$[M+4]^{+*}$	6.06
374	$[M+2]^{+*}$	11.18
372	$[M]^{+*}$	13.64
339	$[M+2]-Cl^{+}$	43.94
337	$[M-Cl]^{+*}$	42.42
297	$[M-SC_3H_7]^{+*}$	21.21
269	$[297-(C_2H_5)+H]^{+*}$	21.21
210	$[(M+4)]-167+H^{+*}$	19.70
208	$[(M+2)-167+H]^{+*}$	74.24
206	$[M-167+H]^{+*}$	57.58
189	$[206-OH]^{+*}$	7.58
167	$[M-206]^{+*}$	25.76
139	$[167-(C_3H_5)+H]^{+*}$	100.00
125	$[167-(C_3H_7)+H]^{+*}$	43.94
97	$[139-(C_3H_7)+H]^{+*}$	86.36

Table 3. Profenofos concentrations found in Farms I, II, III and IV.

Interval (day)	Sampling site	Conc. (ppm) ± SD (n=3)
0 (start of crop cycle)	I	0.75 ± 0.06
	II	0.25 ± 0.05
	III	ND
	IV	1.11 ± 0.25
10	I	0.38 ± 0.05
	II	0.16 ± 0.03
	III	ND
	IV	0.64 ± 0.06
20 (end of crop cycle)	I	0.13 ± 0.05
	II	0.11 ± 0.01
	III	ND
	IV	0.44 ± 0.07

Remark: ND = not detectable

CONCLUSIONS

An organophosphorus insecticide, profenofos, was separated and analyzed by solid-phase extraction and HPLC. The factors affecting the percent recovery of the extraction were studied. The conditions of solid-phase extraction used for the water samples were a 100 ml sample volume, 100 mg sorbent mass, 5 ml of 60% acetonitrile in water as a elution solvent and pump pressure 17 in. Hg. Using these extraction conditions, the average recovery of profenofos was 90%. We found that the farms' drain age water was continually contaminated by profenofos with a concentration range from 0.11 to 1.11 ppm. Although these levels from the plantations were low on average until the time for harvesting and water was kept and circulated within the farms if run-off or flooding occurred, the toxic pesticides could spread rapidly and contaminate these areas. From US legislation, The EC maximum residue limit of profenofos in maize is 0.05 ppm and LC50 (96 hr) for fish is 80-300 µg/L[1]. When compared with their toxicity, the determined level of profenofos in this work was considered high. The aquatic organisms in the study sites could be at high risk from toxic pesticides in water. The risk assessment and prevention of pollution from overuse of pesticides could be considered in the near future. However, this work was to demonstrate the analysis method using SPE following HPLC and MS techniques which provided an accessible approach to measure pesticide residues in water. This study has experimented on the pre-concentration conditions for organophosphorus insecticides, including analysis techniques that could be applied in other, related pesticide determinations.

ACKNOWLEDGMENTS

This work was funded by the Environment for Better Life Foundation of the TPI group and the Graduate School, Chulalongkorn University. The authors are grateful for technical assistance from the Chemical Analysis Service Centre, Hertfordshire University for mass spectral data analysis. The authors thank Ciba-Geigy (Thailand) who kindly donated standard profenofos for our study. The standard mass spectrum from a library search program was provided by the Chemistry Department, Faculty of Science, Chulalongkorn University. The use of chromatographic equipment, facilitated by the Science and Technology Research Equipment Centre, Chulalongkorn University, is greatly appreciated.

REFERENCES

1. Richardson, M. L. and Gangolli, S. (1996) Dictionary of Substances and Their Effects, England Vol. **2**: 460-462; Vol. **5**: 304-309; Vol. **6**: 259-417.
2. Agricultural Regulatory Division, Department of Agriculture, Ministry of AgriculTure Annual Report (1994).
3. Capel, P. D., Ma, L., Schroyer, B. R., Larson, S. T., and Gilchrist, T. A. (1995) Analysis and detection of the new corn herbicide acetochor in river water and rain. *Envi. Sci. Tech.* **29,** 1702-1705.
4. Okumura T. and Nishikawa Y. (1995) Determination of organophosphorus pesticides in environmental samples by capillary gas chromatography - mass spectrometry. *J. of Chromatography.* **709**, 319-331.
5. Buchheit A. J. and Witzenbacher M. (1996) Pesticide monitoring of drinking water with the help of solid-phase extraction and high performance liquid chromatography - atmospheric pressure chemical ionisation mass spectrometry. *J. of Chromatography A* **712**, 85-93.
6. Quayle, W. C., Jepson, I. and Fowlis, I. A. (1997) Simultaneous quantitation of sixteen organochlorine pesticides in drinking water using automated solid-phase extraction, high volume injection, high resolution gas chromatography. *J. of Chromatography A* **773**, 271-276.
7. McLafferty, F. W. (1980) Interpretation of Mass Spectra, 3rd ed. 86-90, University Science Books.
8. Stenhagen, E., Abrahamsson, S., and McLafferty, F. W. (1974) Registry of Mass Spectral Data. 3358, Wiley Publishers.

Received: April 3, 2001
Accepted: May 3, 2001