

Determination of Nine Organophosphorus Pesticides in Cereals and Kidney Beans by Capillary Gas Chromatography with Flame Photometric Detection

Bo Tang^{1,*}, Jin-E. Zhang¹, Li-Guo Zang¹, Yao-Zhong Zhang², Xiang-Yang Li², and Li Zhou²

¹College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014 and

²Institute for the Control of Agrochemicals of Shandong Province, Jinan 250100, China

Abstract

A method is developed for the determination of nine organophosphorus pesticide residues in cereals and kidney beans by capillary gas chromatography with flame-photometric detection. In this method, dichloromethane is used for clean-up after liquid-liquid extraction. It is shown that good separations are obtained using a fused-silica capillary column (DB-1701) by the optimized temperature program. In the spiked levels of 0.012–0.43 mg/kg, the recoveries are from 83.7% to 107%, with the relative standard deviation between 3.2% and 13% and limits of detection from 8.2 to 15 µg/kg. The method is rapid, sensitive, and practical.

Introduction

Cereals and vegetables can be infested by a variety of insects and other pests, which cause a reduction in the quality and quantity of those products. Many of these are controlled by organophosphorus pesticides (OPPs). However, it is known that OPPs form residues in some foods, especially cereals and vegetables (1). Methods for the determination of residues of some OPPs can be found in Chinese national standards (2), and publications are available for the determination of OPPs in wines, medicinal plants, etc. (3–18).

Some new OPPs have been used in conjunction with developments in industry and agriculture. Ethoprop and isofenphos-methyl have been permitted for use in cereals and vegetables in China in recent years. However, the maximum residue limits (MRLs) of the two OPPs have not yet been determined in some countries, including China (19). Therefore, there is a need for a rapid and reliable method to determine the residual levels in cereals and vegetables. Some routine methods used in pesticide residue analysis are often time- and solvent-consuming, although modern trends in analytical chemistry have led to

the simplification and increased automation of preliminary analytical operations, particularly in extraction steps.

A rapid and reliable multiresidue method is described for determining nine organophosphorus insecticides widely used in cereals and vegetables by capillary chromatography, including ethoprophos and isofenphos-methyl, which have not appeared in recent reports. Dichloromethane was used for clean-up after acetone–water had been used for extraction. Nine OPPs fortified in cereals and kidney beans were determined by capillary gas chromatography (cGC)–flame photometric detection (FPD). The method was rapid, simple, and reliable.

Experimental

Reagents and materials

All solvents were of pesticide-residue-analysis grade and were obtained from various suppliers. Acetone and dichloromethane were supplied by Guangcheng Chemical Corp (Tianjing, China). Hexane, acetic ether, and sodium chloride were purchased from Taixing Reagent Factory (Tianjing, China). Analytical standards with certified purities ranging between 90.4% and 99.8% were purchased from the Institute for the Control of Agrochemicals of the Agricultural Department (Beijing, China). Standard solutions containing nine different insecticides were prepared in acetone and stored at 4°C (methamidophos, ethoprop, phorate, diazinon, chlorpyrifos, parathion-methyl, malathion, parathion, and isofenphos-methyl). Different working standard solutions (0.10–10.83 µg/mL) were prepared by dilution in the acetone. Their purities and standard solutions are listed in Table I.

Anhydrous sodium sulfate dried at 400°C for 10 h was supplied by Taixing Reagent Factory (Tianjin, China). Filter aid (Celite 545) was supplied by Shanghai Chemical Corp., China Medical Group (Shanghai, China).

* Author to whom correspondence should be addressed: email tangb@sdu.edu.cn.

GC

Experiments were carried out using a GC-14A system (Shimadzu, Tokyo, Japan) equipped with an FPD and split-splitless injector connected to N2000 GC ChemStation (Zhida, Zhejiang, China). Chromatographic separation was achieved by using a fused-silica capillary column (30-m \times 0.32-mm i.d.) coated with 14% cyano propyl phenyl polysiloxane (0.25- μ m film thickness) (DB-1701) (Hewlett-Packard, Palo Alto, CA). The injector and detector were operated at 240°C and 270°C, respectively. The sample (1.00 μ L) was injected in the split mode at the ratio of 20:1 (1 min). The oven temperature was programmed as follows: 80°C for 1 min, rising to 170°C (15°C/min),

Table I. Purities and Concentrations of Pesticides in Standard Solutions (in Acetone)

Pesticide	Purity (%)	Concentration (μ g/mL)
Methamidophos	99.0	5.362
Ethoprophos	96.5	6.190
Phorate	98.0	2.437
Diazinon	97.5	4.641
Chlorpyrifos	99.8	5.055
Parathion-methyl	98.5	5.895
Malathion	94.3	4.285
Parathion	99.0	5.325
Isofenphos-methyl	90.4	4.433

Table II. Recoveries Obtained in Different Extractants and Eluents

	Solvent	Recovery (%)
Extractant	Acetone	75.8
	Acetone-water (2:1, v/v)	99.7
	Acetic ether	68.9
Eluent	Dichloromethane	73.1
	Sodium chloride-dichloromethane	101
	Hexane	67.8

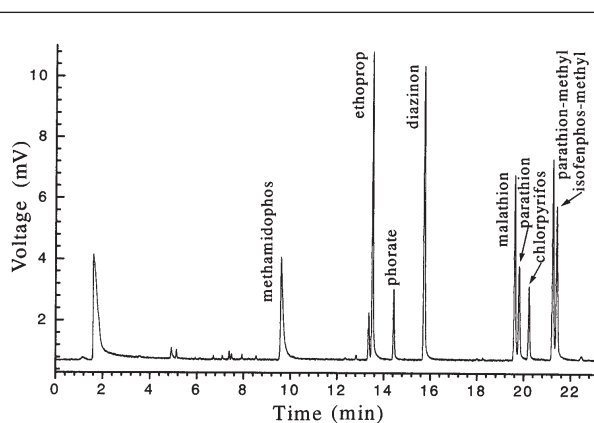


Figure 1. Chromatogram of nine OPPs in standard solution.

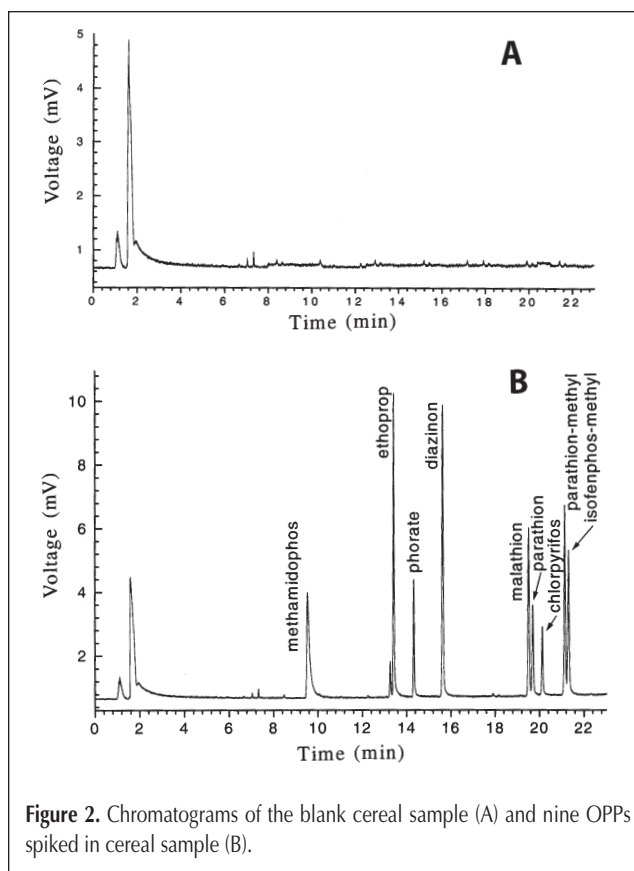


Figure 2. Chromatograms of the blank cereal sample (A) and nine OPPs spiked in cereal sample (B).

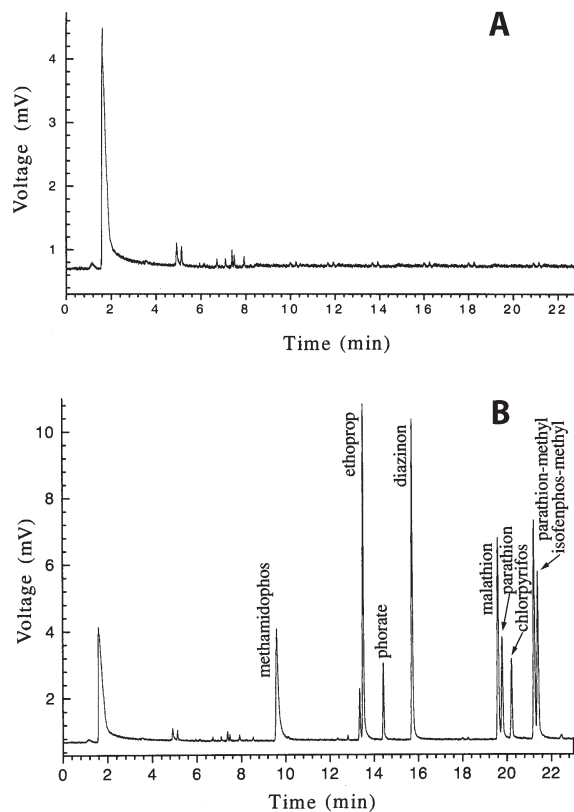


Figure 3. Chromatograms of the blank kidney bean sample (A) and nine OPPs spiked in kidney bean sample (B).

and finally to 240°C (5°C/min) for 2 min. Nitrogen was used as the carrier gas at a constant flow rate of 1.5 mL/min. The make-up gas was also nitrogen at 30 mL/min. Hydrogen was used as the burning gas at a constant flow rate of 90 mL/min. The auxiliary burning gas was air at 60 mL/min.

Sample extraction

Cereals (25.000 g) or kidney beans (50.000 g) were homogenized with 50 mL distilled water and 100 mL acetone for 2 min using a Turrax macerator (IKA, Germany) at 10,000 rpm. The homogenate was filtered through a layer of 10-g filter aid and

the filter cake was rinsed three times with 20 mL acetone. The supernatant was decanted into a 500-mL separating funnel.

Clean-up

The mixtures were heavily shaken for 3 min after 15 g sodium chloride and 50 mL dichloromethane were mixed with the filtrate obtained. They were then kept still and the first organic phase was obtained. Dichloromethane (50 mL) was added into the water phase, and the mixtures were again shaken for 2 min (the second eliminating water). The mixtures were then delaminated again. At this time, the organic phase was lower. The first and second organic phase were, respectively, filtered into the same funnel packed with a little degreased cotton and 30 g anhydrous sodium sulfate. The filter cake was rinsed for three times with 40 mL dichloromethane. The combined filtrates were rotary evaporated (40°C) to a volume of approximately 1 mL, and the final volume of the samples was adjusted in a volumetric flask to 5.0 mL with acetone.

Quantitation and identification

Quantitation was carried out using calibration curves with seven levels, which covered the appropriate concentration range from 0.012 to 0.43 mg/kg. The formula of the quantitation was:

$$c = \frac{A_x \cdot c_s \cdot V}{A_s \cdot m} \quad \text{Eq. 1}$$

where c is the determined value of the pesticide residue in samples mg/kg; c_s is the concentration of the standard solutions in $\mu\text{g/mL}$; V is the last volume of the samples in mL; A_x and A_s are the peak area of samples and standards in $\text{pA} \cdot \text{s}$, respectively; and m is the sample's quantity in g.

Identification was based on the retention time of the nine OPP standards.

Result and Discussion

Optimization of extraction and clean-up

Acetone and water were used to extract on the basis of the pesticides' physical and chemical characteristics, and according to the literature (2). In order to eliminate the water in the extraction, the samples were saturated with sodium chloride and cleaned-up twice with di-

Table III. Linearity of Nine OPPs in 25.00 g of Cereals ($n = 3$)

Pesticide	Concentration (mg/kg)							Correlation coefficient (ρ)
	1	2	3	4	5	6	7	
Methamidophos	0.054	0.11	0.16	0.22	0.27	0.33	0.38	0.9979
Ethoprophos	0.062	0.12	0.18	0.25	0.31	0.37	0.43	0.9965
Phorate	0.024	0.049	0.073	0.097	0.12	0.15	0.17	0.9960
Diazinon	0.059	0.12	0.18	0.24	0.29	0.35	0.41	0.9949
Chlorpyrifos	0.050	0.10	0.15	0.20	0.25	0.30	0.35	0.9910
Parathion-methyl	0.053	0.11	0.16	0.21	0.27	0.32	0.37	0.9998
Malathion	0.043	0.086	0.13	0.17	0.21	0.26	0.30	0.9915
Parathion	0.045	0.089	0.13	0.18	0.22	0.27	0.31	0.9957
Isofenphos-methyl	0.044	0.089	0.13	0.17	0.22	0.26	0.31	0.9983

Table IV. Linearity of Nine OPPs in 50.00 g of Kidney Beans ($n = 3$)

Pesticide	Concentration (mg/kg)							Correlation coefficient (ρ)
	1	2	3	4	5	6	7	
Methamidophos	0.027	0.53	0.80	0.11	0.13	0.16	0.19	0.9977
Ethoprophos	0.031	0.061	0.092	0.12	0.15	0.18	0.21	0.9965
Phorate	0.012	0.024	0.036	0.049	0.061	0.073	0.085	0.9960
Diazinon	0.029	0.059	0.088	0.12	0.15	0.18	0.21	0.9949
Chlorpyrifos	0.025	0.051	0.076	0.10	0.13	0.15	0.18	0.9908
Parathion-methyl	0.026	0.053	0.080	0.11	0.13	0.16	0.19	0.9978
Malathion	0.021	0.043	0.064	0.086	0.11	0.13	0.15	0.9911
Parathion	0.022	0.045	0.067	0.089	0.11	0.13	0.15	0.9957
Isofenphos-methyl	0.022	0.044	0.066	0.089	0.11	0.13	0.16	0.9986

Table V. Recoveries of Nine OPPs Spiked in Cereals ($n = 6$)

Pesticide	Spiked level (mg/kg)	Mean recovery (%)	RSD (%)
	1, 2, 3	1, 2, 3	1, 2, 3
Methamidophos	0.054, 0.27, 0.38	89.8, 92.1, 92.4	8.2, 6.1, 5.8
Ethoprophos	0.062, 0.31, 0.43	90.1, 93.0, 92.8	7.6, 5.4, 8.7
Phorate	0.024, 0.12, 0.17	92.3, 95.2, 95.3	11, 4.5, 4.6
Diazinon	0.059, 0.29, 0.41	90.9, 91.7, 92.9	8.1, 7.1, 5.2
Chlorpyrifos	0.050, 0.25, 0.35	87.6, 90.0, 90.7	6.2, 8.3, 4.9
Parathion-methyl	0.053, 0.27, 0.37	99.8, 107, 103	10, 6.8, 2.0
Malathion	0.043, 0.21, 0.30	97.9, 101, 101	11, 4.2, 3.7
Parathion	0.045, 0.22, 0.31	96.5, 97.2, 98.1	7.0, 5.8, 3.2
Isofenphos-methyl	0.044, 0.22, 0.31	87.4, 90.1, 90.3	9.0, 5.6, 3.7

Table VI. Recoveries of Nine OPPs Spiked in Kidney Beans ($n = 6$)

Pesticide	Spiked level (mg/kg)	Mean recovery (%)	RSD (%)
	1, 2, 3	1, 2, 3	1, 2, 3
Methamidophos	0.027, 0.13, 0.19	88.7, 90.1, 91.0	10, 6.9, 6.1
Ethoprophos	0.031, 0.15, 0.21	83.7, 84.3, 85.7	8.1, 6.4, 8.5
Phorate	0.012, 0.061, 0.085	91.5, 92.0, 91.8	9.6, 5.3, 5.9
Diazinon	0.029, 0.15, 0.21	89.6, 89.9, 88.8	8.7, 8.2, 5.7
Chlorpyrifos	0.025, 0.13, 0.18	90.5, 90.8, 90.9	7.8, 8.6, 6.9
Parathion-methyl	0.026, 0.13, 0.19	93.8, 95.7, 95.6	12, 7.2, 4.1
Malathion	0.021, 0.11, 0.15	91.7, 92.9, 91.4	13, 5.6, 3.8
Parathion	0.022, 0.11, 0.15	92.8, 94.1, 94.3	7.5, 6.4, 3.7
Isofenphos-methyl	0.022, 0.11, 0.16	90.9, 91.2, 91.8	9.2, 6.0, 4.2

Table VII. Retention Time and LOD of Nine OPPs

Pesticide	Retention time (min)	LOD ($\mu\text{g}/\text{kg}$)	
		cereal	kidney bean
Methamidophos	8.96	8.2	11
Ethoprophos	12.72	14	9.7
Phorate	13.58	9.4	15
Diazinon	14.88	12	13
Chlorpyrifos	18.64	14	12
Parathion-methyl	18.82	15	10
Malathion	19.28	10	11
Parathion	20.25	13	9.6
Isofenphos-methyl	20.42	11	12

chloromethane. Recoveries obtained in different extractant and eluent are listed in Table II.

Separation

The described method was carried out to determine the residues of nine OPPs in cereals and kidney beans at the same time. The chromatograms are shown in Figures 1–3, respectively. The nine OPPs were separated thoroughly and the blank samples had no interferences.

Linearity

Different concentrations of nine OPPs were spiked in 25.00 g of cereals and 50.00 g of kidney beans in triplicate, respectively. The correlation coefficients were obtained by peak areas and pesticides' concentrations. The data are shown in Tables III and IV.

Method accuracy, precision, and limit of detection

The accuracy of the method was assessed by the determination of mean recoveries at different limits of quantitation, mid range, and 80% of highest standard in triplicate.

The mean recoveries of nine OPPs spiked levels in samples and relative standard deviations (RSDs) are shown in Table V and VI, respectively. The retention times and limits of detection (LOD) are shown in Table VII.

Conclusion

A rapid, simple, cheap, and efficient clean-up method has been developed for the determination of OPP residues in cereals and kidney beans.

Acknowledgments

This work was supported by the Key Project of National Natural Science Foundation of P.R. China (No. 20335030) and the Key Project of Natural Science Foundation of Shandong Province in China (No. Z2003B01).

References

1. W.K. Zhong, J. Hao, M.X. Sun, and Y.L. Chen. The problem of pesticide pollution in foods in Chinese. *Pesticide (Chin.)* **39(7)**: 1–4 (2000).
2. Chinese Standard Publishing House, 1st Editing Office. *The Comprehensive Compilation of National Standard for Pesticide Residues*. Chinese Standard Publishing House, Beijing, China, 1999, p 176.
3. C. Sala, O. Busto, and J. Guasch. Quick gas chromatographic method for determination common pesticides in musts and wines. *Chromatographia* **44(5-6)**: 320 (1997).
4. M.J. Nunes, M.F. Camões, and J. Fournier. Analysis of organophosphorus, organochlorine and pyrethroid insecticides in medicinal plants. *Chromatographia* **44(9-10)**: 505–13 (1997).
5. F.J. López, J. Beltran, M. Forcada, and F. Hernández. Comparison of simplified methods for pesticide residue analysis use of large-volume injection in capillary gas chromatography. *J. Chromatogr. A* **823**: 25–33 (1998).
6. H.R. Yoon, S.Y. Cho, J.B. Yoon, M.K. Park, and J.H. Park. Analysis of multi-component pesticide residues in herbal medicines by GC-MS with electron impact ionization and with positive- and negative-ion chemical ionization. *Chromatographia* **49(9-10)**: 525 (1999).
7. J.S. Frank and J.L. Steven. Does further clean-up reduce the matrix enhancement effect in gas chromatographic analysis of pesticide residues in food? *J. Chromatogr. A* **868**: 51–61 (2000).
8. Y. Zhang, Z.Q. Huang, and Y.J. Li. Multiresidue determination of organophosphorus pesticides in tea by gas chromatography. *Chin. J. Chromatogr.* **19(3)**: 273–75 (2001).
9. D.H. Kim, G.S. Heo, and D.W. Lee. Determination of organophosphorus pesticides in wheat flour by supercritical fluid extraction and gas chromatography with nitrogen-phosphorus detection. *J. Chromatogr. A* **824**: 63–70 (1998).
10. L.S. Ana and V.B. Luís. Validation of a solid-phase microextraction method for the determination of organophosphorus pesticides in fruits and fruit juice. *J. Chromatogr. A* **833**: 35–42 (1999).
11. R. Serrano, F.J. López, and F. Hernández. Multiresidue determination of persistent organochlorine and organophosphorus compounds in whale tissues using automated liquid chromatographic clean up and gas chromatographic-mass spectrometric detection. *J. Chromatogr. A* **855**: 633–43 (1999).
12. J. Oliva, A. Barba, N. Vela, F. Melendreras, and S. Navarro. Multi-residue method for the rapid determination of organophosphorus insecticides in grapes, must and wine. *J. Chromatogr. A* **882**: 213–20 (2000).

13. K.N.T. Norman and H.W.P. Sean. Supercritical fluid extraction and quantitative determination of organophosphorus pesticides residues in wheat and maize using gas chromatography with flame photometric and mass spectrometric detection. *J. Chromatogr. A* **907**: 247–55 (2001).
14. M. Fernández, C. Padrón, L. Marconi, S. Ghini, R. Colombo, A.G. Sabatini, and S. Girotti. Determination of organophosphorus pesticides in honeybees after solid-phase microextraction. *J. Chromatogr. A* **922**: 257–65 (2001).
15. Ch. Lentza-Rizos, E.J. Avramides, and F. Cherasco. Low-temperature clean-up method for the determination of organophosphorus insecticides in olive oil. *J. Chromatogr. A* **912**: 135–42 (2001).
16. M.T. Tena, A. Rios, M. Valcárcal, and M. Sánchez-Alarcón. Supercritical fluid extraction of organophosphorus pesticides from orange samples: effect of solid additives on recovery. *Chromatographia* **46(9-10)**: 524–28 (1997).
17. H.R. Yoon, E.J. Lee, M.K. Park, and J.H. Park. Sulfuric acid pretreatment for the simultaneous GC screening of organochlorine and organophosphorus pesticides in herbal essential oils. *Chromatographia* **47(9-10)**: 587–92 (1998).
18. K. Szymeczyk and M. Malczewska. Gas chromatography analysis of organophosphorus pesticides in plant samples. *Chromatographia* **48(1-2)**: 156–57 (1998).
19. W.J. Zhuang. *The Comprehensive Compilation of National Maximum Residue Limits for Pesticides, Veterinary Drugs in Foodstuffs and Feedstuffs in the World*. China Foreign Economic Relation and Trade Publishing House, Beijing, China, 1995, p. 21.

Manuscript received January 21, 2004;
revision received April 8, 2005.