A New Analysis Method with GC or GC–MS for the Quick Detection of Pesticide Residues in Vegetables

Jianyu Guo and **Xiangmin Zhang***
Department of Chemistry, Fudan University, Shanghai, 200433, P.R. China

Abstract

A new analytical method for gas chromatography (GC) or GC–mass spectrometry (MS) using the direct sampling technique is described. This direct sampling technique, which bypasses the conventional complicated sample pretreatment process, is applicable to cases of fast detection of pesticide residues in foods and large-scale screening of samples by portable GC in field detection. By a direct sampling technique, the vegetable sample is ground into paste, and 30 mg is placed directly into the evaporating chamber for GC–MS identification and quantitation (by full-scan mode). The GC column used is an HP-5 (30.0-m × 250-µm × 0.25-µm, 5% phenyl methyl siloxane). Chlorpyrifos, bromophos, fenpropathrin, γ-666, and pp'-DDT are chosen to represent organophosphorus, pyrethrins, and organochlorine pesticides because they are chief objects of the detection of pesticide residues in vegetables. Rape, a common and mass-consumed vegetable in China, is chosen as the sample in this study. The detection limits for these pesticides by the full-scan mode are all below the maximum pesticide residue limit of vegetables set by the Ministry of Agriculture of China, and the reproducibility of this method is acceptable. This analysis method is proven to be simple, quick, and reliable and is suitable for multipesticide residues analysis of vegetables. It can also be used in the analysis of vegetable components and signal chemicals.

Introduction

Intensive agriculture implies the use of large quantities of pesticides on many vegetable crops. To control pesticide residue levels within the maximum residue levels (MRLs) (1), various analysis methods have been developed. Generally, analytical methods for pesticide residues in foods require multiresidue analysis at high recoveries (> 70%) accompanied by low detection limits and a simple procedure (2). Traditional laboratory analysis methods are especially good with respect to accuracy and sensitivity but can be complex and time-consuming. For some occasions such as field detection and quick screening of foods from a marketplace for high pesticide residues, simple and quick analysis methods are required.

Traditional laboratory analysis procedures can be generalized as follows: (*i*) sample extracted by organic solvent (acetonitrile or acetone are usually used) using food agitator or ultrasonic oscillator (3–6); (*ii*) purified by column chromatography; (*iii*) concentrated; (*iv*) separated and detected by high-performance liquid chromatography using UV detection or mass selective detection (MSD) (7,8) or by gas chromatography (GC) using electron capture detection, nitrogen phosphorous detection, atomic emission detection, MSD (1,9–11), etc. Of the entire process, the first step is the most complicated and time-consuming. Therefore, for field use, the main focus of improving the analysis method is on the aspects of miniaturization and simplification of the sample extraction and cleanup procedure. Some modified techniques for sample treatment have been reported, such as solid-phase extraction, matrix solid-phase dispersion, supercritical fluid extraction (12–15), etc. Some biological techniques aimed at fast screening have also been reported (16–18), but they still require the positive samples to be sent for further analysis in a laboratory.

The purpose of this study was to develop a simple, fast, and also accurate analysis method for the screening and determination of pesticide residues in vegetables. In this article, a new direct sampling technique for GC or GC–MS was developed by bypassing the traditional, complicated sample pretreatment procedure. This method proved to be quick, universal, and accurate, as well as requiring less and also organic solvent. The method also works well for rapid detection of pesticide residues in foods and large-scale screening in field detection. This method can also be used in the analysis of vegetable components and signal chemicals.

Experimental

Chemicals and reagents

Chlorpyrifos, bromophos, fenpropathrin, g-666, and pp'-DDT standard samples (purity > 99%) were purchased from the Agro-environment Protection Institute, Ministry of Agriculture (MOA) of China (Tianjin, China). Methanol was of analytical reagent grade.

Stock solutions of each pesticide were prepared in methanol at 1 mg/mL and were stored in a refitted refrigerator (4°C). Each standard solution was prepared by appropriate diluting of the stock solution with methanol and was stored in a refitted refrigerator (4°C).

* Author to whom correspondence should be addressed

Sample pretreatment

First, 2 kg of blank rape sample (without pesticide) or positive rape sample (sprayed with pesticide standard solution) was cut to pieces, then the pieces of the sample were collected and homogenized by a food agitator. Samples (50 g) from the homogeneous mixture were ground to a paste with a mortar and pestle before use.

Direct sampling technique

The carrier gas was shut down at first; the nut on the top of the evaporating chamber was twisted off, and the inside glass liner tube was taken out for direct sample introduction. To make standard curve, first, variable concentrations of pesticide standard solutions were injected manually onto the middle of the glass liner tube inner wall at a 1 µL injected volume. Second, to make quantitative a determination of pesticide residue in rape sample, a 30-mg pretreated rape sample was put into the middle part of the glass liner tube inner wall directly by a home-made small spoon, and the paste sample was carefully spread on the inner wall.

When the sampling procedure in the glass liner tube was ready, the glass liner tube was reinstalled for analysis. The carrier gas was set at a flow rate of 1.0 mL/min. In order to remove most of the methanol in the sample, the carrier gas mode was first set in the split mode for 1 min before analysis at the split ratio of 100:1. After 1 min, the mode was changed to splitless, and the analysis program on GC–MS began at this time.

When the analysis process was over, the dry rape sample was taken out of the glass liner tube inner wall, and the tube was washed in turn by methanol, chromic acid solution ($K_2Cr_2O_7$–H_2SO_4), water, and methanol again, then oven-dried for the next sampling.

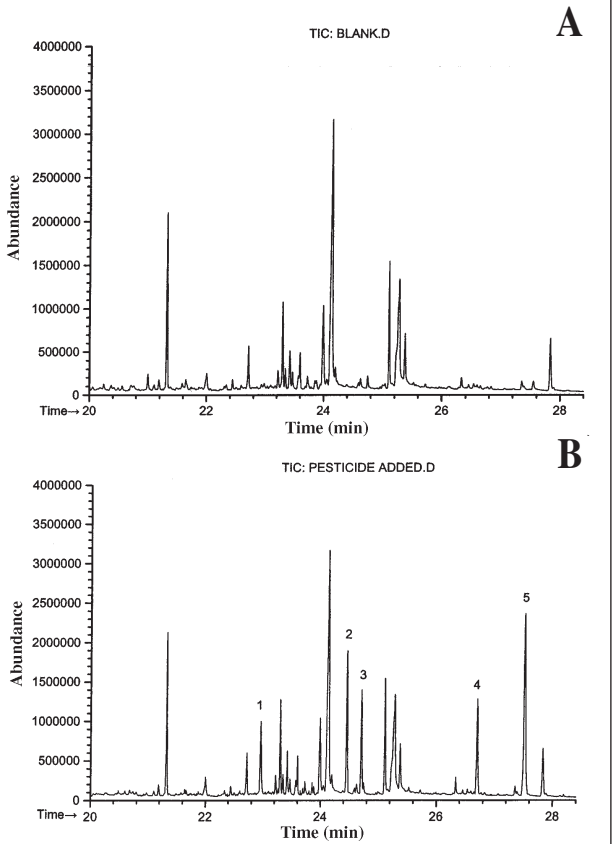

Figure 1. TIC of the rape samples without pesticide (A) and with five pesticides (B) is shown. The identities of the rape are as follow: 1, γ-666 (22.96′, 2.6mg/kg); 2, chlorpyrifos (24.46′, 2.9 mg/kg); 3, bromophos (24.71′, 2.8mg/kg); 4, pp′-DDT (26.70′, 2.7mg/kg); and 5, fenpropathrin (27.52′, 2.9 mg/kg).

GC–MS

The GC–MS system consisted of a Hewlett-Packard 6890 GC with a 5973 MSD (Palo Alto, CA). The GC was equipped with an HP-5MS capillary column (30-m × 0.25-mm id) coated with a 0.25-µm film of stationary phase (polydimethylsiloxane containing 5% phenyl), and the carrier gas was helium (99.999%) at a flow rate of 1.0 mL/min. The temperature program used for analysis was as follows: the initial temperature was 40°C, held for 11 min, then raised to 270°C at 15°C /min and held for 30 min. The injector temperature holding at 40°C was raised directly to 240°C in 7 min, held for 3 min, and then the heating stopped.

The samples were ionized by electron impact mode with electron energy of 70 eV. The ion source temperature was set at 230°C. A full-scan acquisition mode (*m/z* 35–500) was used in this study. All compounds found in the samples were identified by comparing their mass spectra with those in the National Institute of Standards and Technology library (Washington, DC) and also by comparing the GC retention time with those of standard samples.

Results and Discussion

Optimization

In this study, the injector temperature was set at 40°C and ramped up to the final temperature of 240°C. When the final tem-

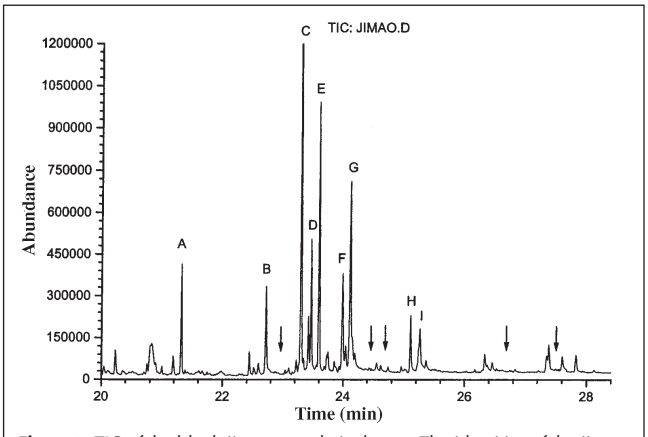

Figure 2. TIC of the blank jimao sample is shown. The identities of the jimao components are as follows: A, (E)-stilbene; B, tetradecanoic acid; C, 3-tetradecyne; D, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-; E, 9-octadecyne; F, Z-7-hexadecenoic acid; G, *n*-hexadecanoic acid; H, phytol; I, 9,12,15-octadecatrienal. The arrows are pointing to the positions of the five pesticides referred to in the text if present, which indicates that no matter what the vegetable is, its components do not interfere with the detection of pesticides.

perature was set lower (~ 180–235°C), the recoveries of the pesticides were too low (< 70%); though above 245°C, some pesticides (such as bromophos and chlorpyrifos) appeared to decompose. Therefore, 240°C was selected as the best final hold temperature. Holding this temperature for 3 min guaranteed that the pesticide sample was completely volatilized and well-concentrated at the head of the cold column.

In this study, chlorpyrifos, bromophos, fenpropathrin, γ-666, and pp'-DDT were chosen as to represent organophosphorus, pyrethrins, and organochlorine pesticides for the following reasons; as far as vegetables are concerned, chlorpyrifos, bromophos, and fenpropathrin are the most frequent pesticides found in common use. Although γ-666 and pp'-DDT were forbidden 20 years ago, the residues still remain in the soil because of their very slow degradation rate. Therefore, these pesticides are usually the chief objects of the detection of pesticide residues in vegetables.

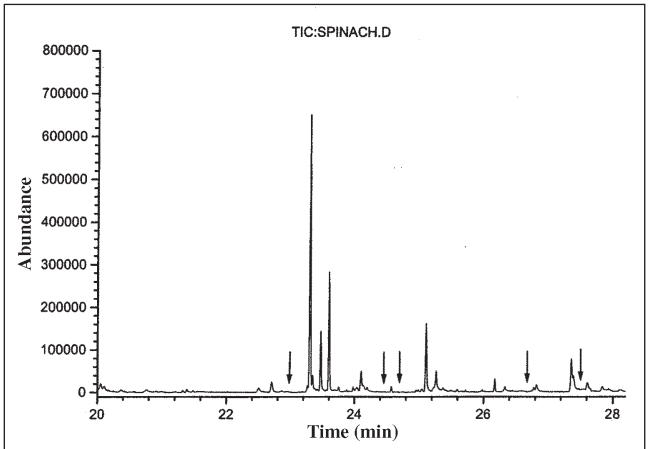

Figure 3. TIC of the blank spinach sample is shown. The arrows are pointing at the positions of the five pesticides referred to in this article if present.

It is necessary that the minced vegetable sample be ground to paste before GC–MS analysis. The grinding process can make the pieces of the minced vegetable sample more homogeneous, so that the analysis result can be more representative; the experimental results showed that the recoveries for the pesticides were greatly enhanced after the vegetable sample pieces were ground.

This analysis method proved to be simple, quick, and reliable and especially suitable for the analysis of thermally stable and chemically inert pesticides. There were some pesticides (methamidophos and omethoate) that showed bad chromatograms when sampled by the title technique, and the recoveries were also not satisfactory. It can be interpreted that by the sampling method described here, the temperature sensitive pesticides (such as omethoate) can be partially decomposed in the evaporating chamber, and some chemically active pesticides (such as methamidophos) can also react with some components of plants during the heat process in the evaporating chamber.

Table I. The Linear Regression Equations for Calibration of the Five Pesticides

Pesticide	t_R (min)	Linear regression	Linear concentration range (μg/mL)	r*
Chlorphyrifos	24.46	$Y = -2.25 \times 10^7 + 2.54 \times 10^7 X$	10–1000	0.9997
Bromophos	24.71	$Y = -1.88 \times 10^7 + 2.11 \times 10^7 X$	10–1000	0.9995
Fenpropathrin	27.52	$Y = -2.34 \times 10^7 + 2.63 \times 10^7 X$	10–1000	0.9999
γ-666	22.96	$Y = -1.09 \times 10^7 + 1.25 \times 10^7 X$	10–1000	0.9996
pp'-DDT	26.70	$Y = -1.50 \times 10^7 + 1.69 \times 10^7 X$	10–1000	0.9993

* Correlation coefficient.

Table II. The Recovery, Precision, and Detection Limit for the Five Pesticides (n = 3)

	Avg. recovery (%)			RSD*			
	Addition level (mg/kg)			Addition level (mg/kg)			Detection limit
Pesticide	3.0	1.5	0.3	3.0	1.5	0.3	(mg/kg)
Chlorphyrifos	88.05	91.42	102.62	1.9	2.0	2.7	0.11
Bromophos	67.41	70.54	103.82	2.6	3.1	3.8	0.15
Fenpropathrin	99.58	99.70	101.11	1.8	2.1	2.5	0.10
γ-666	93.55	95.27	106.76	7.7	8.2	10.9	0.12
pp'-DDT	96.33	93.83	109.38	8.7	9.4	16.6	0.13

* Relative standard deviation.

Separation

Under the operating conditions described previously, the pretreated rape sample without pesticide or with the five previously mentioned pesticides was directly injected onto the glass liner tube inner wall for GC–MS analysis under full-scan mode. The total ion chromatograms (TIC) are shown in Figure 1 (A and B). Changing the vegetable sample to others, such as spinach, jimao, water spinach, etc., resulted in chromatograms similar to that found for rape (Figures 2 and 3). The primary peaks in these chromatograms can usually be assigned to phytol, squalene, long chain alkane, carbene, and plant acid. At the GC–MS operating conditions described previously, these peaks (common in the plants) are observed to be well separated from those of the pesticides in the chromatograms of the positive samples and do not interfere with the detection of pesticides.

Quantitation

Standard curve

Determinations were carried out under full-scan mode. The content of pesticide residue in rape sample was quantitated according to its peak area. To make calibration curves, each standard solution of pesticide using variable concentrations (n = 5) were analyzed three times, and the average peak area was used. Linear regression analysis was performed on these data, and these regression equations are shown in Table I. In the table, Y is the peak area and X is the logarithm of the concentration of the pesticide solution.

Precision, recovery, and detection limit

The spike recovery method was used in the precision and recovery tests. Standard solutions of the five pesticides were added in the blank rape samples (without pesticide) at additional levels of 3.0, 1.5, and 0.3 mg/kg. Rape samples were prepared as described previously and analyzed under the GC–MS operating conditions using the direct sampling technique. The data of the precision and recovery tests are shown in Table II.

The recoveries of chlorpyrifos and bromophos were lower than the other three pesticides in Table II. This can be attributed to chlorpyrifos and bromophos being more sensitive to heat than the other three pesticides, and the two pesticides can be partially decomposed at the injection temperature.

It was noted that there were three small peaks at approximately the right time to be bromophos, pp'-DDT, and fenpropathrin (Figure 1). These can be assigned to be some components of plants, and they were also observed in the chromatograms of spinach, jimao, water spinach, etc. (Figures 2 and 3). When the pesticide concentrations in the sample are high enough, these small peaks do not significantly influence the quantitation. However, with the reducing of the pesticide concentrations in the sample, the influence of the interference peak become obvious, and the recoveries of the pesticides appeared to be increasing accordingly because of the integrating error (Table II). For example, at the concentration of 0.3 mg/kg, the recovery of pp'-DDT was found to be 109% (Table II), which cannot be considered to be as accurate as those found at higher concentrations. If the quantitative analyses of the pesticides were carried out in the selected ion mode (SIM) instead of the full-scan mode, the interference should be removed. However, SIM is only applicable for GC–MS but not for GC with other detectors.

The detection limits (s/n = 5) of the five pesticides by the full-scan mode were all below 0.15 mg/kg (see Table II), which are below the MRL of vegetables set by the MOA of China (1). At the concentrations of the detection limits, these pesticides can still be detected by the MS detector but cannot be quantitated accurately.

The reproducibility of this method proved to be acceptable (Table II). This method especially fits for rapid detection of pesticide residues in foods and large-scale screening for the positive vegetables in field detection.

Determination of pesticide residues in vegetables from a market

Thirty-eight batches of rape from a market in Shanghai, China were sampled randomly. Determination of pesticide residues was performed in these samples using the direct sampling technique reported here. In one batch, chlorpyrifos residue was found at a concentration of 1.03 mg/kg (Figure 4), which is above the MRL set by the MOA of China.

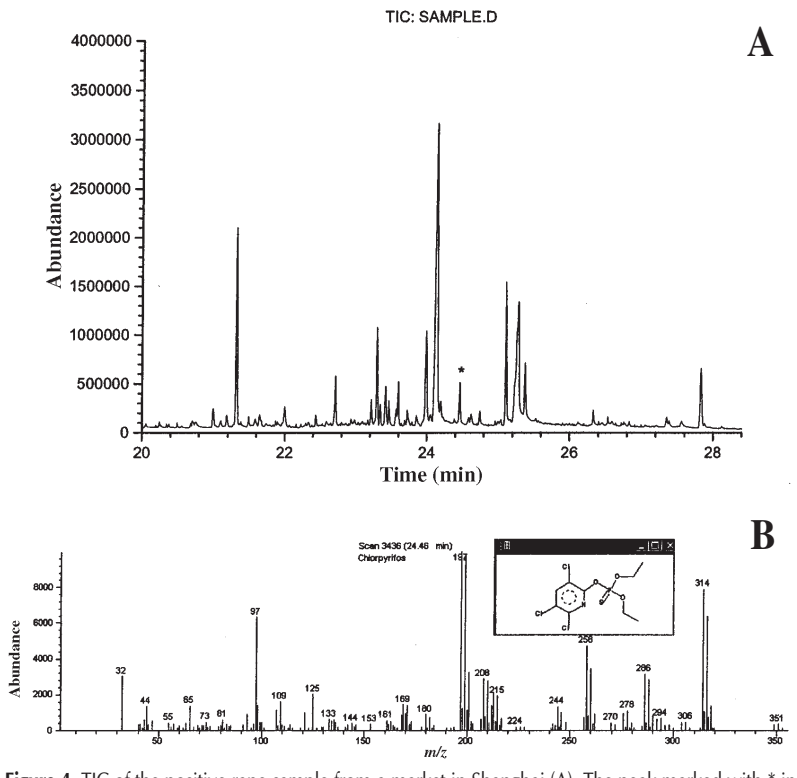

Figure 4. TIC of the positive rape sample from a market in Shanghai (A). The peak marked with * in A is assigned to chlorpyrifos according to its mass spectrum (B).

Conclusion

The analysis method using the direct sampling technique has the advantages of being simple, quick, and reliable and proved to be suitable for the determination of multipesticide residues in vegetables, especially for rapid detection and large-scale screening in the field. It can be concluded that this method can also be used in the analysis of plant components and signal chemicals. Study in this field will continue in the future.

Acknowledgments

We gratefully acknowledge the support of the National Science Foundation of China for this work.

References

1. C.M. Torres, Y. Picó, and J. Mañes. Determination of pesticide residues in fruit and vegetables. *J. Chromatogr. A* **754:** 301–31 (1996).
2. National Pesticides Residues Research Group. *Handbook Of Practical Methods For The Determination Of Pesticide Residues,* Vol. 2 (in Chinese). Chemical Industry Press, Beijing, P.R. China, 2001.
3. A. Aguera, M. Contreras, and A.R. Fernandez-Alba. Gas chromatographic analysis of organophosphorus pesticides of horticultural concern. *J. Chromatogr. A* **655:** 293–300 (1993).
4. G.F. Pang, C.L. Fan, Y. Z. Chao, and T.S. Zhao. Packed-column gas-chromatographic method for the simultaneous determination of 10

pyrethroid insecticide residues in fruits, vegetables, and grains. *J. AOAC Int.* **77:** 738–47 (1994).
5. V. Lopez-Avil, J. Benedicto, and K.M. Bauer. Stability of organochlorine and organophosphorus pesticides when extracted from solid matrixes with microwave energy. *J. AOAC Int.* **81:** 1224–32 (1998).
6. V. Lopez-Avil, R .Young, and W.F. Beckert. Stability of organic pollutants during microwave-assisted extraction from solid matrixes. *J. AOAC Int.* **81:** 462–76 (1998).
7. T. Tomsej and J. Hajjslova. Determination of benzoylurea insecticides in apples by high-performance liquid chromatography. *J. Chromatogr. A* **704:** 513–17 (1995).
8. C.H. Liu, G.C. Mattern, X. Yu, and J.B. Louis. GC/MS and LC/MS determination of 20 pesticides for which dietary oncogenic risk has been estimated. *J. Agric. Food Chem.* **39:** 700–704 (1991).
9. W.M. Draper. Optimizing nitrogen-phosphorus detector gas chromatography for pesticide analysis. *J. Agric. Food Chem.* **43:** 2077–82 (1995).
10. K.C. Ting and K. Peng. GC MIP AED method for pesticide-residue determination in fruits and vegetables. *J. AOAC Int.* **74:** 991–98 (1991).
11. Y. Yamazaki and T. Ninomiya. Determination of bitertanol residues in strawberries by liquid chromatography with fluorescence detection and confirmation by gas chromatography mass spectrometry. *J. AOAC Int.* **81:** 1252–56 (1998).
12. A. Sosa, A. Lenardón, and M.L. Mattassini. Screening of pesticides via solid-phase extraction and gas chromatography. *J. Chromatogr. Sci.* **41:** 92–95 (2003).
13. J. Kuivinen and S. Bengtsson. Solid-phase extraction and cleanup of organophosphorus pesticide residues in bovine muscle with gas chromatographic detection. *J. Chromatogr. Sci.* **40:** 392–96 (2003).
14. K. Fodor-Csorba. Chromatographic methods for the determination of pesticides in foods. *J. Chromatogr. A* **624:** 353–67 (1992).
15. J. Sherma. Pesticides. *Anal. Chem.* **67:** R1–R20 (1995).
16. H. Schulze, E. Scherbaum, M. Anastassiades, S. Vorlova, R.D. Schmid, and T.T. Bachmann. Develop-ment, validation, and application of an acetylcholinesterase-biosensor test for the direct detection of insecticide residues in infant food. *Biosen. Bioelectron.* **17:** 1095–105 (2002).
17. I.A. Toscano, G.S. Nunes, and D. Barcelo. Immunoassays for pesticide analysis in environmental and food matrices. *Food Technol. Biotechnol.* **36:** 245-55 (1998).
18. A.D. Lucas, S.J. Gee, B.D. Hammock, and J.N. Seiber. Integration of immunochemical methods with other analytical techniques for pesticide-residue determination *J. AOAC Int.* **78:** 585–91 (1995).

Manuscript received November 19, 2003;
revison received December 14, 2004.