

A Survey of Determination for Organophosphorus Pesticide Residue in Agricultural Products

Wen Li, Ming Sun and Minzan Li

Key Laboratory of Modern Precision Agricultural System Integration Research of Education, China Agricultural University, China
No. 17 Tsinghua East Road Haidian District, Beijing, 100083, P.R. China

Abstract: In order to find a fast, high efficient determination method of Organophosphorus Pesticides (OPPs) residue because OPPs widely used in crops pest control fields in China are causing fearful risks for environment as well as animals and human health, traditional and advanced determination methods were discussed in the study. Based on the spectrum analysis technology combined colorimetric OPPs residue detection experiments in leafy vegetables showed that the absorbance of color reaction between OPPs residues and suitable colorimetric reagents can be distinguished in ppm level of OPPs residues. The detection limit of chlorpyrifos after color reaction with 0.5% $PbCl_2$ in acetic acid solution is 0.5 ppm. The conclusion was drawn that the detection technologies were diversified, however, a simple, efficient, rapid and nondestructive detection method is lacking and the spectrum analysis technology combined colorimetric can be a new fast and efficient determination method in the future.

Keywords: Colorimetric, determination method, OPPs, spectrum analysis

INTRODUCTION

Organophosphorus Pesticides (OPPs) were invented at the end of 1930s as the second generation synthetic pesticides with characteristics of broad spectrum, high efficiency, many varieties and short residual toxicity period. Now there are mainly methamidophos, parathion-methyl, chlorpyrifos, dimethoate, omethoate, Malathion, chlordimeform, Furadan, etc., often sprayed on fruits and vegetables in China. If OPPs residues in fruits and vegetables or in other environment enter into organism, most of them have inhibitory action on cholinesterase in organism, that is to say, the cholinesterase will accumulate because of its losing ability to decompose acetylcholine, then will make nerve function disorder to lead to the body damage. Therefore, it is extremely vital significance to grasp pesticide residue status in agricultural products, reduce pesticide residue level and protect consumers' physical health by detecting pesticide residue timely. In recent years, more and more strict requests for OPPs residue limits have been put forward by governments all over the world, which makes the detection difficulty increase.

Advantages and disadvantages of traditional and advanced detection methods are studied in detail in the study and it reflects the diversification of detection means and the interdisciplinary application of advanced technologies in order to seek a fast, efficient detection method for OPPs residues.

Colorimetric is a classic chemical detection method based on color reactions which can make the object to be detected conveniently in visible wave band, but its sensitivity and accuracy is poor if only used to detect OPPs; Spectrum analysis is a simple, rapid and nondestructive detection technology, but it has unstable performance, poor precision to meet the quantitative requirement if directly used to detect OPPs. The authors researched OPPs residues in leafy vegetables based on the spectrum analysis technology combined colorimetric and discovered that ppm level OPPs residues can be distinguished by the absorbance values of color reaction with suitable colorimetric reagents. The method can provide us help for seeking a fast, efficient detection method.

METHODOLOGY

Physicochemical methods: According to the toxicology and chemical properties of OPPs, primary detection methods of OPPs are wave spectrum, chromatography and chromatography-mass spectrum and so on.

Wave spectrum is a kind of qualitative or quantitative (or limited) determination method based on specific wavelength color reaction produced by chemical reactions (such as oxidation, sulfonation, esterification and complexation etc.) which can be produced between some functional groups or hydrolysate, redazate of OPPs and color reaction of

special wavelength in certain conditions. Microchemistry experiment method used in the former was low sensitive and tended to produce false negative interfered by many factors during the experiment. The OPPs contains different perssads which would produce different chemical reactions. In order to improve detection sensitivity the microchemistry experiment method was replaced gradually by wave spectrum (OPP were detected by spectrophotometer etc.). The wave spectrum can only detect one kind of OPPs or OPPS contains the same perssads at one time. Samples contain different OPPs residues are detected roughly with low accuracy and positive samples need to be determined again by chromatography and mass spectrometry.

Chromatography is widely used in OPPs detection nowadays. It is divided into three categories: TLC (Thin-Layer Chromatography), GC (Gas Chromatography) and LC (Liquid Chromatographic) according to the physical and chemical characteristics in the process of detection. TLC is a more mature and widely applied trace detection method. Firstly the determined OPPs are extracted with suitable solvent, then purified and concentrated, finally separated and expanded in a thin layer of silicon sheet, so the determination can be detected qualitatively by comparing Rf (Rate of flow) value to the standard OPPs or detected by thin-layer canner. Compared with wave spectrum, Chromatography is more economic, simple and rapidly but not accurate. Its detection limit is generally 0.1-0.01 µg. GC is a new device analysis method develops based on the column chromatography since the 1950s and has been widely used. Through extraction, purification and concentration, OPPs are poured into gas chromatographic column. Gasified by temperature program, different OPPs in fixed phase are separated and detected by entering into different detectors to be scanned by corresponding gas chromatograms to draw gas chromatogram. GC can determine the chemical composition of OPPs based on the remained time of the gas chromatograms and the quantity by peak height or peak area comparing with standard curve. GC is accurate and its threshold is merely 1ng. Xiong *et al.* (2009) determined 13 OPPs residues in vegetables based on capillary double column GC with FPD (Flame Photometric Detector). The samples were extracted directly with acetonitrile, then salted out and concentrated, finally separated with DB-1701 and HP-50+ capillary column. Kang (2010) used domestic and imported gas chromatography but got different detection results when using different pesticides or in different concentration. They also got different gas chromatograph linear range when using different pesticides. They concluded domestic

Table 1: Color reagents of three pesticides and their properties

Color reagents	Color	Sensitivity	OPPs type
Pbcl ₂	Yellow	5-10 µg	Chlorpyrifos, rogor, phoxim
Resorcin	Red	1-5 µg	Chlorpyrifos
Na ₂ Fe (CN) ₅ NO	Purple-red	5-10 µg	Rogor

instrument couldn't meet the requirements because GC needed highly accuracy. HPLC (High-Performance Liquid Chromatography), develops by improving gas chromatography theory, is a chromatographic analysis method based on liquid phase column chromatography. Lacorte *et al.* (1998) got a detection limit of 13-105 ng/L based on parathion-methyl, tiguron, Diazinon and Dursban by HPLC-DAD technology. However, LC instruments are too expensive to be popularized in a large scale.

Chromatography-Mass Spectrum combines GC or LC with mass spectrometry, it can detect pesticide residue qualitatively and quantitatively because it has the advantage of high separation efficiency of chromatography and the feature of appraisal accuracy for compound structure of mass spectrometry, so it is particularly suitable for pesticides metabolites, degradation products and especially multi-residues analysis. The detection threshold of the method is generally 0.001 mg~0.01 mg/kg. HPLC-MS is used to detect pesticides and compounds with high molecular weight, polarity or intense unstable thermal. Chen *et al.* (2008) detected 8 pesticide residues in agricultural products, such as soybean, tomato and onion, by GC-MS. All the results met the requirements of quantitative analysis. Markoqlou (2007) detected 5 BPU pesticides in tomatoes based on LC-MS at the same time and got detection limit of 0.008-0.01 mg/L which was only 1/10 of the residue threshold of the international regulation. Compared with GC, LC is more efficient, highly sensitive, rapidly and widely used. However, chromatography-mass spectrum instrument is too expensive to be used widely in pesticide residues detection.

Colorimetric can determine the analyte content by comparing or measuring material solution color depth, which is widely used as a conventional detection method, but its application in pesticide residues detection have not yet relevant research reports. As shown in Table 1, experiments shows that chlorpyrifos, rogor, phoxim can react with several color reagents according to the components of them, but the sensitivity is poor.

Rapid methods: In recent years, Enzyme Inhibition Method (EIM), immunoassay and biosensor technology have been researched at home and abroad in order to obtain a rapid, sensitive, accurate and simple detection

method. In addition, the spectral analysis as a rapid, easy and nondestructive detection technology in pesticide residues becomes highly hotspot.

EIM is based on toxicology characteristics of OPPs which can inhibit acetyl cholinesterase activity. In the absence of OPPs, acetylcholine can produce choline and acetic acid by the action of acetyl cholinesterase, cholinesterase is inhibited while OPPs exist, cholinesterase catabolite, acetic acid, can reduce accordingly. OPPs can be detected according to indicator color or PH value of reaction liquid. Qiu *et al.* (2010) detected OPPs in 5 kinds of vegetables with acetyl cholinesterase extracted from crucian brain, liver and muscles. Wang *et al.* (2009) detected residues of OPPs and Carbamic acid ester (CBs) with acetyl cholinesterase based on EIM. Akkad and Schwack (2008) analyzed OPPs with three kinds of esterase and achieved better results. EIM is rapid, easy operation and low cost, but the enzyme reagent tends to lose activity and cause the reaction unstable. So the detection results based on EIM have big errors and poor repeatability with the confirmation rate at about 60-70% in the practical application.

Immunoassay is a kind of specific sensitive detection technology develops based on the principle of the specificity combination of antigen and corresponding antibody in vitro. Pesticide residues analysis use of IA is mainly divided into two aspects: Radioactive Immune Analysis (RIA) and Enzyme leauge Immune Analysis (EIA), EIA develops rapidly and has now become one of the most widely used technologies. ELISA (enzyme-linked immunosorbent assay) used in pesticide residues has developed comprehensively and rapidly since the 1990 s. IA, GC and LC are listed as the three pillars of the pesticide residues analysis technology by the ACS (American Chemical Society). Kumar *et al.* (2006) detected parathion-methyl in the environment and the food by the combination of EIA and FIA (Flow Injection Analysis), the results showed its high sensitivity and satisfactory specificity. Liu *et al.* (2009) found a kind of monoclonal antibody named D12-B5 which had significant specificity and favorable effect in detecting OPPs such as parathion-methyl, Dursban, Tiguron and fenitrothion etc. But IA has very strong specificity: one kind of kit can only detect a single kind of OPPs rather than multi-residues. The detection of similar structure compounds often has some cross detection results, in addition the antigen is not extracted easily and the kit cost is high.

Biological sensor is usually considered as an analysis tool that can select and responded reversibly to

certain kinds of compounds or biological active substance when a kind of biological sensitive component tied to a converter closely. After the determinant combines with molecular recognition elements specifically, which consist of the biological function substances with recognition ability such as enzyme, microbe, antigen and antibody etc., light and heat produced from the above specific combination were transform to the electrical signals and light signals which can be output through a signal converter. The electrical signals and light signals are displayed or recorded with instruments and processed by electronic technology. According to the different sensitivity of biological sensors to biological molecular recognition elements, biological sensors can be classified as the enzyme biosensor, immune biological sensors, microbial sensor and the bionic biological sensors, etc. The research of Istamboulie *et al.* (2009) showed that phosphotriesterase had the possibility of decomposing some OPPs, so a new enzyme biosensor applied in the selective detection of chlorfenvinphos and Dursban had been developed. Mauriz *et al.* (2006) detected chlorpyrifos in water with a SPR (Surface Plasmon Resonance) immune sensor, firstly chlorpyrifos artificial antigens were fixed on the sensor gold membrane surface then compete with the detached chlorpyrifos to combine in the determinant. The increase of chlorpyrifos concentration led to the decrease of SPR signals. ppt levels of pesticide residues can be detected using method and the SPR immune sensor has a good stability even to be used repeatedly for 200 times. However, biological sensor technology is still at the beginning stage and there are some disadvantages such as poor stability, short service life, etc.

Spectrum Analysis (SA) is a simple and rapid detecting technology and studies the molecular structure and dynamic characteristics based on the interaction of light and matter to obtain the chemical information related with samples by getting information of the launch, absorption and scattering of light. Xue *et al.* (2008) discussed the detection method of pesticide residues on fruit surface by hyper spectral imaging technology using navel orange as the research object, analyzed the hyper spectral images by PCA (Principal Component Analysis), finally found out the characteristic wavelengths in the images. With the characteristic images obtained by image processing technology it can detect higher concentration pesticide residues on fruits surface, but for low concentration pesticide residues it can't be recognized precisely. Zhou *et al.* (2004) had a test for common fruits and pesticides

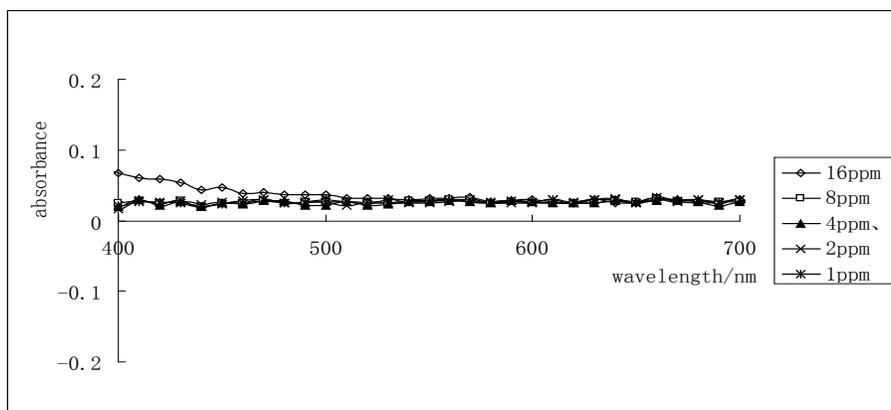


Fig. 1: Absorbance of different concentration of chlorpyrifos without color reactions

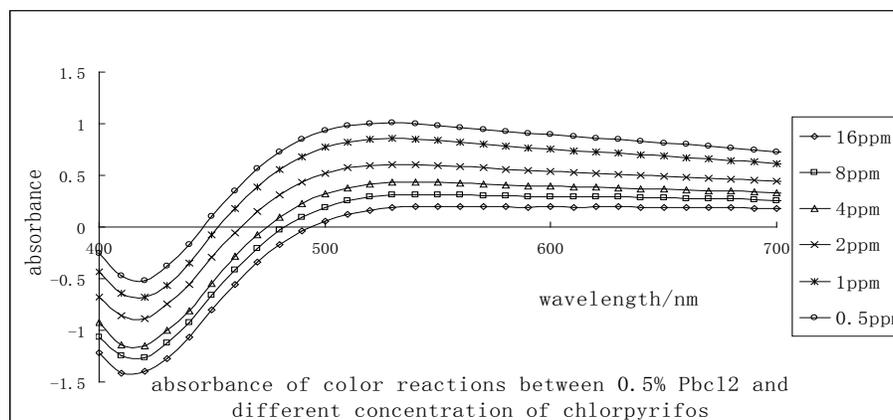


Fig. 2: Absorbance of color reactions for different concentration of chlorpyrifos

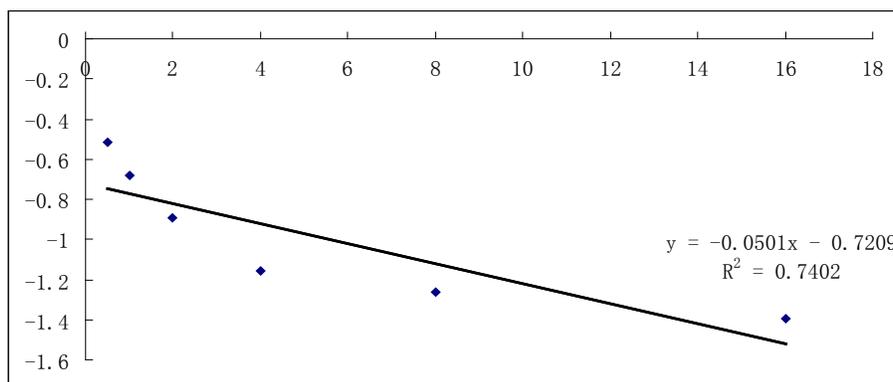


Fig. 3: Regression equation of absorbance of color reactions for different concentration of chlorpyrifos at 420 nm absorbance peak

with a near-infrared Fourier transform Raman spectrometer with the excitation wavelength of 1064 nm, the results showed that characteristic Raman spectra were successfully recorded respectively. Li *et al.* (2004) investigated the mid-infrared Attenuated

Total Reflection spectra of two slathered pesticides dichlorvos and trichlorfon in vegetable juice solution. It can be concluded that pesticides in standard solution and vegetable juice solution have almost the same absorbance characteristics. The model built by the

absorbance data pesticides in water solution to simulate their absorbance in vegetable solution, pesticide residues on vegetables can be detected directly based on infrared spectrum technology. It provides a possible way to detect pesticide residues in vegetables rapidly and qualitatively. Chen *et al.* (2010) developed a hyper spectral fluorescence imaging system to trace chemicals in vegetable samples using hyper spectral imaging technology combined with fluorescence analysis. The experiment results showed that chlorpyrifos had strong fluorescence characteristic. The emission spectrum of chlorpyrifos indicated that the peak emission wavelength is 437 nm, but chlorpyrifos samples with different concentration have different fluorescence emission spectral intensity at the peak and fluorescence emission peak value reduces when the concentration of the chlorpyrifos decreases.

Although spectrum analysis is a rapid detection technology, it has unstable performance, poor precision to meet the quantitative requirement if directly used to detect OPPs. The authors firstly detected the absorbance of chlorpyrifos of 0.5, 1, 2, 4, 8 and 16 ppm, respectively without color reactions, the absorbance couldn't be distinguished as shown in Fig. 1, then studied respectively after color reactions with 0.5% PbCl₂ in acetic acid solution and experiments showed that the absorbance of chlorpyrifos and its concentration was negatively correlated, as shown in Fig. 2.

The line shown in Fig. 3 is fitted by the method of least squares at 420 nm characteristic absorption peak.

When 40 chlorpyrifos samples with 0.5-20 ppm were detected by spectrophotometer UV-2450, the regression equation is $y = -0.6901 - 0.0528x_{420}$, the coefficient of correlation $R = 0.9578$. It means the spectrum analysis combined colorimetric is helpful for OPPs residues detection.

Advanced methods: Chemiluminescence (CL) technology has developed as a high sensitive detection technology for organophosphorus trace residue in recent years. Huang and Wang (2003) detected pesticide residue in food by using CL combined with immunoassay, molecular engram and micro fluidic chips, etc. The bionic biological sensor made by molecularly imprinted polymer and BLM (Bilayer Lipid Membrane) also interested the researchers. In addition, CL technology may become rapid, sensitive and simple if the new luminous agent synthesis combined with other technologies (such as micro fluidic chip technology, sensor technology, etc.). A research team (Mihoubi *et al.*, 2008) in University of Southampton in England developed a miniature semiconductor laser source emitting a 480 fs pulse at the wavelength of 1044 nm and with the laser source all-semiconductor room-temperature terahertz time domain spectrometer was manufactured, it is of

important significance for pesticide residues detection research based on the THZ spectrum technology.

CONCLUSION

In a word, OPPs residues detection technique at home and abroad is diversified. Using combined detection methods has become the development trend of the technology. However, so far a rapid, efficient and simple detection method has not been found to meet the increasing detection standard, especially the field detection. According to experiments, spectrum analysis combined colorimetric is helpful to detect OPPs residues. Therefore, it has very important theoretical and practical value to research and develop a new rapid, efficient detection technology for OPPs residues.

ACKNOWLEDGMENT

This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (No. 201003008) and the Project Supported by National Research Center of Intelligent Equipment for Agriculture.

REFERENCES

- Akkad, R. and W. Schwack, 2008. Multi-enzyme inhibition assay for detection of insecticidal organophosphates and carbamates by high-performance thin-layer chromatography. *JPC J. Planar Chromat.*, 21(6): 411-415.
- Chen, J., Y. Peng, Y. Li, W. Wang, J. Wu and J. Shan, 2010. Rapid detection of vegetable pesticide residue based on hyperspectral fluorescence imaging technology. *Trans. CSAE*, 26(S2): 1-5.
- Chen, Y., X. Lu, H. Zhao, M. Huang, C. Xu and J. Wang, 2008. Simultaneous detection of pesticide residues from seed coating in agricultural products using GC-MS. *J. Instr. Anal.*, 27(10): 1084-1087.
- Huang, Z. and J. Wang, 2003. The detection of dichlorvos pesticide by chemiluminescence method. *J. Qinghai Norm. Univ. Nat. Sci.*, 1: 59-63.
- Istamboulie, G., D. Fournier and J.L. Marty and T. Noguer, 2009. Phosphotriesterase: A complementary tool for the selective detection of two organophosphate insecticides: Chlorpyrifos and chlorfenvinfos. *Talanta*, 77(5): 1627-1631.
- Kang, W.J., 2010. Application of gas chromatography for organophosphorus pesticide residue detection in vegetables. *Hunan Agric. Sci.*, 1: 76-78.
- Kumar, M.A., R.S. Chuhan and M.S. Thakur, B.E.A. Rani, Bo. Mattiasson and N.G. Karanth, 2006. Automated flow Enzyme-Linked Immunosorbent Assay (ELISA) system for analysis of methyl parathion. *Anal. Chim. Acta*, 560: 30-34.

- Lacorte, S., J.J. Vreuls and J.S. Salau, F. Ventura and D. Barcelo, 1998. Monitoring of pesticides in river water using fully automated on-line solid-phase extraction and liquid chromatography with diode array detection with a novel filtration device. *J. Chromatogr. A*, 795: 71-82.
- Li, W., K. Xu, Y. Wang, Z. Lei and Z. Zhang, 2004. Investigation on the detection of pesticide residue in vegetable based on infrared spectroscopy [J]. *Spectrosc. Spect. Anal.*, 24(10): 1202-1204.
- Liu, Y., Y. Lou and D. Xu, G. Qian, Q. Zhang, R. Wu, B. Hu and F. Liu, 2009. Production and characterization of monoclonal antibody for class-specific determination of 0,0-dimethyl organophosphorus pesticides and effect of heterologous coating antigens on immunoassay sensitivity. *Microchem. J.*, 93(1): 36-42.
- Markoqlou, A.N., 2007. Determination of benzoylurea insecticide residues in tomatoes by high-performance liquid chromatography with ultraviolet-diode array and atmospheric pressure chemical ionization: Mass spectrometry detection. *J. AOAC. Int.*, 90(5): 1395-1401.
- Mauriz, E., A. Calle, L.M. Lechuga, J. Quintana, A. Montoya and J.J. Manclús, 2006. Real-time detection of chlorpyrifos at part per trillion levels in ground, surface and drinking water samples by a portable surface plasmon resonance immunosensor. *Anal. Chim. Acta*, 561: 40-47.
- Mihoubi, Z., K.G. Wilcox, S. Elsmere, A. Quarterman, R. Rungsawang, I. Farrer, H.E. Beere, D.A. Ritchie, A. Tropper and V. Apostolopoulos, 2008. All-semiconductor room-temperature terahertz time domain Spectrometer. *Optic. Lett.*, 33: 2125-7.
- Qiu, C., X. Liu, H. Ren and F. Jiang, 2010. Study on detection of organophosphorus pesticide residue in vegetables by enzyme inhibition method. *Food Mach.*, 26(2): 40-42.
- Wang, J., F. Tan, L. Wang, K. Duan, H. Zhu, K. Zhao and X. Tang, 2009. Advances on rapid detection of pesticide residues by the method of restraining acetyl cholinesterase activity. *Acta Agric. Shanghai*, 25(4): 131-5.
- Xiong, Y., J. Yang, Y. Kang, Y. Chai, J. Liu, N. Chu and Y. Yang, 2009. Residue determination of 13 organo-phosphorous pesticides in vegetables. *Southwest China J. Agric. Sci.*, 22(2): 528.
- Xue, L., J. Li and M. Liu, 2008. Detecting pesticide residue on navel orange surface by using hyperspectral imaging. *Acta Optic. Sin.*, 28(12): 2277-2280.
- Zhou, X., Y. Fang and P. Zhang, 2004. Raman spectra of pesticides on the surface of fruits. *Chin. J. Light Scatter.*, 16(1): 11-14.