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Food Safety: Monitoring of Organophosphate Pesticide Residues in Crops and Food

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Organophosphate (OP) pesticides are used globally today, to control a wide range of insect pest species in agriculture, homes, and gardens, as well as in veterinary practice. OP pesticide residues in crops, food and food by-products are both a health and international trade issue. At present, QuEChER, matrix solid phase dispersion (MSPD), gel permeation chromatography (GPC), SPE, GC-FPD, GC-NPD, GC-MS, HPLC-MS, and other methods are widely used to detect OP pesticides residue in food. New issues at China Agricultural University (CAU) such as molecular imprinting, matrix effects, storage stabilities and chiral separation of OP pesticides are addressed in this article.

Keywords Analytical methods; organophosphate pesticides; residue

INTRODUCTION

There are more than 40 major organophosphate (OP) pesticides used globally today to control a wide range of insect pest species in agriculture, homes, and gardens, as well as in veterinary practice. OP insecticides exhibit potent acute and/or subchronic toxicity to mammals. Both OP and carbamates insecticides are potent acetylcholinesterase (AChE) inhibitors, and they are the first priority group of pesticides reviewed under the United States Food Quality Protection Act. The United States Environmental Protection Agency (USEPA) began the risk management program with OP insecticides in August 1996. The final draft report on the cumulative risk assessment of OP pesticides was issued

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in August 2006. The Chinese Government has begun to ban the production and restriction of application of 5 OPs (highly toxic, including methamidophos, parathion, etc.).

OP pesticide residues in crops, food, and food by-products [residues exceed the Maximum Residue Levels (MRL)] are both a health and international trade issue. This is a difficult problem—especially the use and MRL of some of the key OP products, which have been withdrawn recently. This article provides an overview of the development of the OP residue analytical method at China Agricultural University (CAU) to monitor OP residues in crops and food in China. The Chinese standard method includes matrix solid phase dispersion (MSPD) or gel permeation chromatography (GPC) cleanup methods. A standard protocol (NY/T 761), originally from US California Department of Food and Agriculture (CDFA), was adopted. The current internationally accepted and widely used “QuEChERS method” was also successfully validated at CAU on GCMS.

Additionally, this paper will discuss the residue analytical method design, GCMS and/or HPLC-MS method validation, and the storage stability of selective OP residues in crops and processed commodities. The OP insecticides residues monitoring status in Chinese vegetables collected in the past several years will be addressed.

RESIDUES ANALYTICAL METHODS FOR OPS IN MONITORING

Extraction, Cleanup, and Detection

At present, QuEChERS, matrix solid phase dispersion (MSPD), gel permeation chromatography (GPC), SPE, GC-FPD, GC-NPD, GC-MS, HPLC-MS etc. methods are widely used to detect OP pesticides in food.

QuEChERS method was published by Anastassiades and Lehotay et.al. In this method the dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, and sugars, to some extent from the food extracts.¹ This current internationally accepted and widely used “QuEChERS method” was also successfully validated at CAU on GC-MS, and special attention is paid for leek, onion alike samples cleanup.

MSPD and gas chromatographic method for simultaneous detection of five OP pesticides residues (dichlorvos, methamidophos, omethoate, malathion, parathion) in concentrated apple juice was established by Jian-ke Li. The recoveries of $0.1 \text{ mg} \cdot \text{kg}^{-1}$ and $1 \text{ mg} \cdot \text{kg}^{-1}$ are $\geq 96\%$ with RSD $1.4\% \sim 9.9\%$ and $\geq 87\%$ with $2.8\% \sim 8.5\%$ respectively, the minimum

detectable levels are $0.007\sim0.025\text{ mg}\cdot\text{kg}^{-1}$. The method is faster, easier, and more inexpensive than liquid-liquid extraction.²

Tobacco sample was extracted with acetone; the resultant extract was cleaned by liquid-liquid partition with ethyl acetate and cyclohexane, and then purified by GPC. The residues of 10 OP pesticides were determined by GC-FPD. The average recoveries of the method were ($72.8\%\sim108.1\%$) and RSD was less than 10% .³

The residues of 24 OP pesticides in *Ganoderma* were determined by Ji-wei Lu et.al. Pesticides were extracted by ultrasonication in ice bath, then cleaned-up with GPC. The residues were simultaneously identified and quantified with dual-column and dual-flame photometric detector (FPD). The average recoveries and RSD were in the range of $75.28\%\sim117.18\%$ and $3.19\%\sim15.57\%$, respectively.⁴

A gas chromatography for simultaneous determination of 11 kinds of OPs in fruits and vegetables was developed by Ren-qun Zhu et.al. OP pesticides were extracted from fruits and vegetables with acetone used as an extractant, and contents were measured with NPD detector.⁵

A SPE-HPLC-MS method for the determination of methamidophos, monocrotophos, trichlorphon, malathion, parathion, diazion and phoxim in vegetables has been developed by Yuan-jin Xu. The fragmentation patterns of the seven OP pesticides were summarized by the fragment ion of MS.^{2,6}

Fast Screening Method

Purified acetylcholinesterase (AChE) was made from *Musca domestica* by Procainamide-Based affinity chromatography. OP pesticides could be rapid detected by the color developed during enzymatic reaction and the detecting sensitivity is $0.001\text{ mg kg}^{-1}\sim0.1\text{ mg kg}^{-1}$. The method is convenient and time-saving.⁷ However, the limitation of AChE inhibition method is false positive or false negative.

Molecularly Imprinted Polymers

Molecularly imprinting utilizing synthetic material to mimic natural receptors as a recognition element to provide selectivity and sensitivity against target molecules for affinity-based separations, biomimetic receptors, and sensors is becoming an important technique.⁸⁻¹⁰ Molecularly imprinted polymers (MIPs) are polymerized by crosslinking functional monomer(s) onto polymer networks in the presence of a template molecule. Removal of the template reveals binding cavities fitted to the

template. Functional monomers situated in the cavity lead the template to selectively rebind into the cavity again.

An analytical methodology for the analysis of four polar organophosphorus pesticides (monocrotophos, mevinphos, phosphamidon, and omethoate) in water and soil samples incorporating a molecularly imprinted solid-phase extraction (MISPE) process using a monocrotophos imprinted polymer was developed. The recoveries of four polar organophosphorus pesticides (OPPs) extracted from 1 L of river water at a 100 ng/L spike level were in the range of 77.5–99.1%. The recoveries of organophosphorus pesticides extracted from a 5-g soil sample at the 100 $\mu\text{g/kg}$ level were in the range of 79.3–93.5%.¹¹

A method was described for the selective binding behavior of paraoxan and parathion compounds on surface imprinted polymers which were prepared using both charge transfer (CT) (methacryloyl-antipyrine, MAAP) and ligand-exchange (LE) (methacryloyl-antipyrine-gadalonium, MAAP-Gd) monomers.¹²

Spontaneous formation of an ordered nano-TiO₂/p-tert-butylcalix arene hybrid thin film imprinted by parathion has been achieved by self assembled technique, and a sensor based on the imprinted film has been constructed for the selective determination of parathion. A linear response to parathion in the concentration range of 5.0×10^{-8} to 1.0×10^{-5} M was observed with a good correlation coefficient ($\gamma^2 = 0.992$). The linear regression equation was $I_p (\mu\text{A}) = 0.202C (\mu\text{M}) + 0.797$, and the detection limit of the sensor was 1.0×10^{-8} M (S/N = 3). The imprinted film sensor has been applied to the determination of parathion in spiked vegetable samples and the recoveries were varied from 93.3 to 103.3% at 0.3 and 2.0 μM .¹³

MATRIX EFFECTS IN GC WITH OP PESTICIDES DETECTION

Enhancement Effect

Matrix components compete with analytes for active sites on the injector parts and protect susceptible pesticides from adsorption or decomposition (see Figure 1). This matrix effect is called enhancement effect.

In our work, a multi-residue method was developed for rapid determination of 52 pesticides and their isomers in vegetables and fruits by GC-MS in selective ion monitoring mode, with the aid of analyte protectants. Pesticides were extracted from samples with acetonitrile, and the extract was cleaned up using dispersed primary-secondary amine (PSA) absorbent. It was demonstrated that applying protectants could provide a more accurate quantification and compensate “matrix-induced

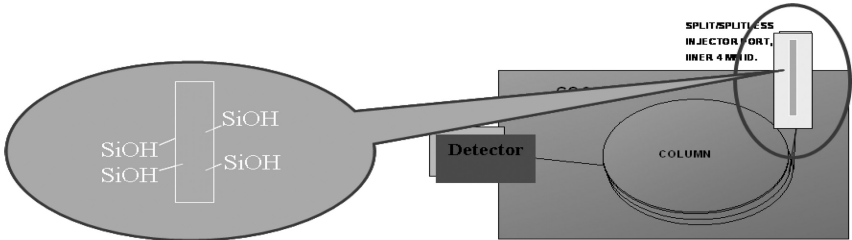


FIGURE 1 The active sites on the injector parts.

chromatographic response enhancement” in gas chromatography-mass spectrum (GC-MS) analysis, thus increase reliability of test.^{14,15}

Effects of three compounds used as protectants on compensation for matrix effect are evaluated in mixed matrix and comparison with matrix-matched standard are made. The matrix-induced error for most pesticides has been shown to be avoided by adding 3-ethoxy-1,2-propanediol (D05, 20 mg/mL) and D-sorbitol (B06, 1 mg/mL) before injection, especially for acephate, omethoate, monocrotophos, methamidophos, paraoxon, etc (See Figure 2).

The concepts of the “relative matrix effect” and “absolute matrix effect” in assessment of matrix- introduced effect in pesticide residues

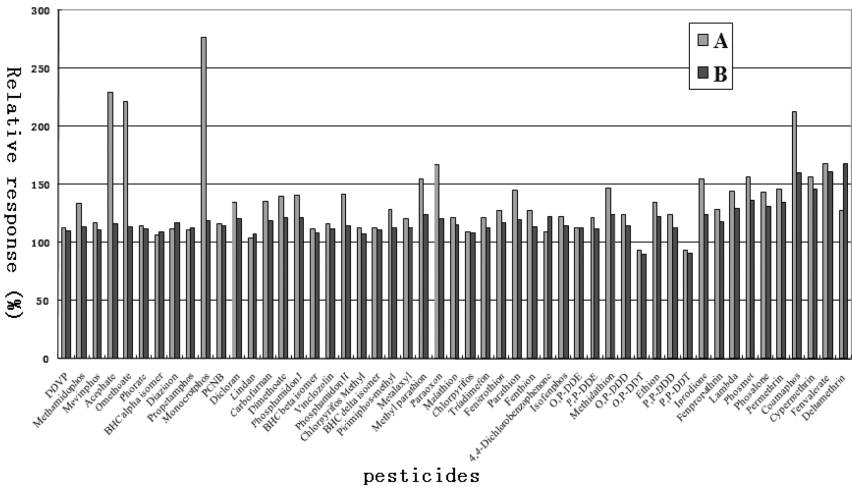


FIGURE 2 Comparison of relative responses of pesticides without (A) and with (B) the analyte protectant. A = peak area in matrix-matched standard/peak area in solvent standard; and B = peak area in matrix-matched standard with protectan/peak area in solvent standard with protectant.

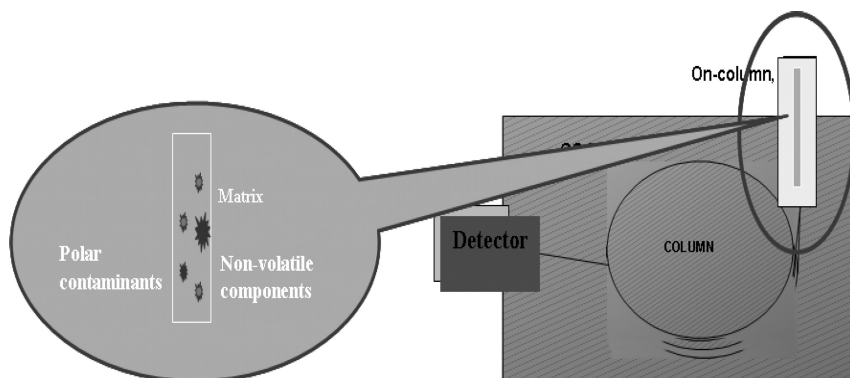


FIGURE 3 Non-volatile matrix components adsorb and deposit analytes.

analysis are introduced, and relative matrix effect about 45 pesticides in 22 produce types are evaluated. Results indicated that pesticides show different variability in certain extent in relative response between different matrixes. Certain pesticides are matrix-dependent such as paraoxon, methamidophos, acephate, and so on. In addition, some pesticides, such as methyl-hlorpyrifos, chlorpyrifos, methyl-pirimiphos, malathion, and so on, show relative high analytical stability in various matrixes.

Diminishing/Suppression effect

Non-volatile matrix components accumulated in the inlet or in the front section of a capillary column from matrix adsorb and deposit analytes in the injector port (See Figure 3). Figure 4 shows the improvement of peak shape and sensitivity by adding protectants in matrix/standard.

STORAGE STABILITIES OF OP PESTICIDES

Stored samples play a significant role in pesticide residue analysis process. Since the beginning of pesticide usage and application on commodities and crops, it is of great importance for residue analysts to know what is happened during storage of pesticides in different matrix. For pesticide registration, people worked more on the stability issues. The main three aspects are stability in the field; during processing or during storage. Limited information was found in literatures and reports about storage stability of pesticide residue in crops and plant extracts.¹⁶ As the storage time is prolonged, many pesticides are likely to be degraded for various reasons that would lead to false results and

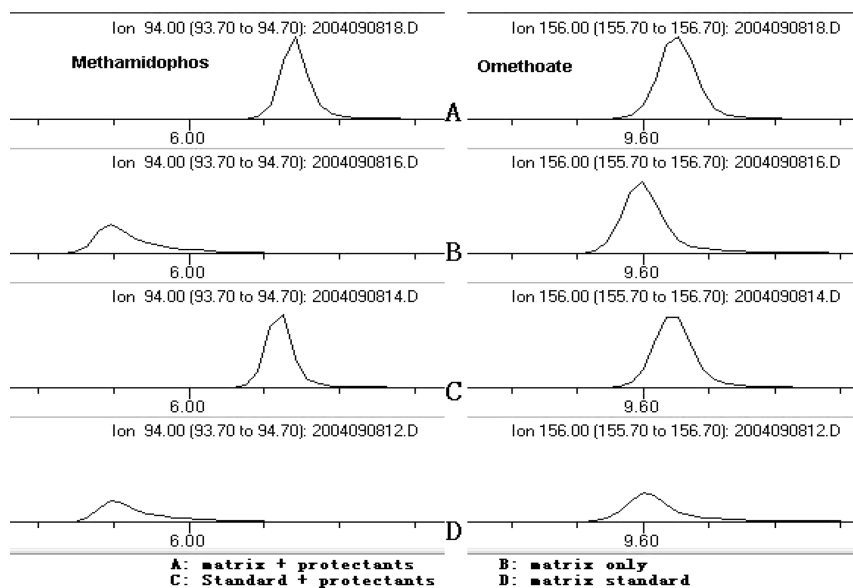


FIGURE 4 The improvement of peak shape and sensitivity by adding protectants in matrix/standard.

affect the experimental accuracy. Therefore, study of storage stability is urgent for insuring how long time the pesticides can still be valid in order to obtain reasonable results.

In our study, thirty commonly used pesticides including insecticide; fungicide and herbicide were studied by fortifying them into five crops (lettuce, orange, cucumber, cabbage, and unpolished rice) during storage for 6 mon at 4°C or -20°C to investigate the effects of storage time of these pesticides in crop samples. The results confirmed that the stability of most of the pesticides stored in five crops during 6 mon period at -20°C, only a few pesticides degraded such as malathion in cucumber; fenthion in cucumber and lettuce; methidathion in orange. Nevertheless, after 6 mon of storage bout one third of tested pesticides had degraded. Significant losses of some of these pesticides were observed only after 1 mon. Usually high water solubility and polar pesticides hydrolyze possibly during long time storage in crops, but some of them do not hydrolyze. Oxidation could sometimes be an important process (e.g., thio-compounds), but generally the rate of oxidation of organic compounds is slow. The pH value of the crop sample had a significant influence on the stability of only these pesticides those acid/basic properties. The initial pesticide played a significant role, the lowest concentrations being the least stable from lettuce and orange fortified with a

low spiking level of 0.5 mg/kg and a high spiking level of 5 mg/kg stored for 6 mon at 4°C or -20°C.

The stability of commonly used pesticides in plant samples was evaluated. Matrices differing in the character of co-extracts were represented by cucumber, tomato, apple, orange, lettuce, and unpolished rice. After the QuEChERS process, spiked filtrates were stored for 20 day at 2°C or 120 day at 4°C. Degradation of pesticide standards in pure acetonitrile solution was studied under the same conditions as in the case of plant extracts. Some degradation of most organophosphates in acetonitrile and all plant extracts after 120 days at 4°C is observed. Most pesticides are stable enough to store plant samples 60 days at 4°C or 10 day at 20°C prior to further handing, or to use them as calibration to avoid matrix-induced enhanced and pressed response.

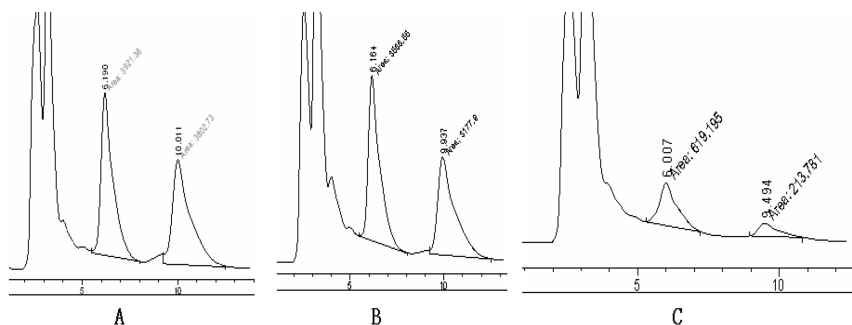
Decompositions of the commonly used pesticides in apple samples during refrigerated storage and room temperature were studied. Apple samples were lowered into the aqueous mixtures of pesticides formulations for 1 min.; kept at room temperature under a fume hood for 24 h, and stored at 4°C or 25°C. Concentration of organophosphates pesticides decreased above 50% after 32 days at 25°C.

The stability of stored pesticide residue is very complicated. The character of pesticide (structure, Kow, water solubility and vapour pressure, and others) play an important role, the pH value, water content, and some pigment of matrix and temperature had significant influence on the stability of compounds. Most pesticides can be stored at -20°C for 6 mon or more (See Table I). But few pesticides are degraded in during a period time of storage. Pesticide in chopped sample was better stability than in full sample.

ANALYSIS AND DEGRADATION OF CHIRAL OP PESTICIDES

The chiral separations of many chiral pesticides were performed and quantitative analysis methods for the enantiomers were developed in our research work. According to the previous references, the systemic investigations on the chiral pesticide enantiomers in this dissertation were not performed before. The methods developed were reliable for the determining the enantiomers in technical material and environmental samples.

Five polysaccharide derivatives including cellulose-tris(3,5-dimethylphenylcarbamate) (CDMPC), amylose-tris(3,5-dimethylphenylcarbamate) (ADMPC), cellulose-tris(phenylcarbamate) (CTPC), amylopectin-tris(phenylcarbamate) (ATPC) and amylose tri- (S)-1-phenylethylcarbamate were synthesized and coated to γ - amino-propylsilica to prepare chiral stationary phases (CSPs). The chiral



A: 0 day; B: 2 day; C: 6day

FIGURE 5 The chromatograms of the enantioselective degradation of isocarbophos enantiomers in soil.

separations of chiral pesticides on the CSPs were performed by high-performance liquid chromatography (HPLC) applying n-hexane, n-heptane, n-pentane, or petroleum ether as mobile phase and ethanol, n-propanol, iso-propanol, n-butanol, or iso-butanol as polar modifier. The influences of alcohols and temperature on the chiral separations were studied. The two enantiomers of malathion, isocarbophos, methamidophos, or profenofos could gain complete separation under optimized condition.

The eluting enantiomers were identified by a circular dichroism (CD) detector. The thermodynamic parameters concerning the chiral separations were calculated based on linear van't Hoff equation.

The validated data including linearity, precision and limit of detection showed that the methods were reliable for determining the optical purity of the technical material and the residual analysis. Furthermore, the residual analysis procedures of the enantiomers of sixteen chiral pesticides in soil and water samples were developed. Acetone and dichloromethane were used to extract the enantiomers from soil samples and solid phase extraction (SPE) with C18 matrix was used to extract them from water. The results showed that the methods were reliable for the residual analysis of the enantiomers in environmental samples.

The enantioselective degradation of isocarbophos and profenofos enantiomers in soil was studied. The (+) - (CD signal) enantiomer degraded faster than the (–)-enantiomer of the three chiral pesticides in soil resulting the higher proportion of the (–)-enantiomer in soil. The half-lives ($t_{1/2}$) of the enantiomers were calculated (See Figure 5).

TABLE I The Loss of OP Pesticides Stored in Five Samples at 4°C or -20°C after 6 mon

Pesticide	4°C (%)					-20°C (%)				
	Lettuc	Orange	Cucumber	Cabbag	Unpolished ric	Lettuc	Orange	Cucumber	Cabbag	Unpolished ric
Omethoate	27.6	45.2	32.4	35.5	63.7	>	>	>	>	>
Monocrotophos	>	56.4	>	>	65.3	>	>	>	>	>
Dimethoate	>	>	>	>	43.4	>	>	>	>	>
Isocarbophos	>	>	>	>	>	>	>	>	>	>
Methidathion	49.5	54.5	50.7	48.1	28	>	39.7	>	>	>
Malathion	70.7	>	104.8	52.8	>	30.5	>	53.1	>	>
Fenitrothion	35	37.5	67.5	>	>	>	>	>	>	>
Diazinon	105.5	84.4	108.6	98	>	>	>	>	>	>
Fenthion	101.8	52.5	98.4	85.7	37.6	40.5	>	40	>	>
Chlorpyrifos	44.5	40	30.4	>	25.6	>	>	>	>	>

≥stable in sample.

CONCLUSION AND DISCUSSIONS

OP pesticides residues have been monitored in China (mainly for vegetables). Analytical methods are internationally equivalent. Around 40 OPs could be monitored by Standard Protocol (based on USDA method). GC-MS method by QuEChERS was adapted. Monitoring data have to be used statistically. Special attention should be paid in analytical labs, these including: stability of storage, matrix effects in chromatography analysis. New technologies: for example, molecular imprinting, GC-MS-MS etc. Enantiomers of Chiral pesticides usually have different biological activity and different toxicology profile. Several chiral OPs have been separated and their environmental behaviors were tested. More need to be done on this field, for instance, more compounds, expanding research of metabolism in the animal tissues. This may concentration on the stable compounds.

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