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Determination of trichlorfon from spicy vegetable samples using molecularly-imprinted solid phase extraction technique

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Abstract : Trichlorfon is a widely used organophosphorus insecticide that has adverse effect to human health. The complexity of matrix encountered in vegetables, especially for those spicy vegetables, makes the screening of trace trichlorfon a discouraging challenge. In this research, molecularly-imprinted solid phase extraction (MISPE) was investigated for the enrichment of trace amount of trichlorfon from ginger, allium fistulosum, onion, garlic and chili samples. Recoveries of 80.2%-89.3%, RSD of 2.5%-3.5% (n=5) and LOD of 0.05 μg/mL were achieved for trichlorfon by using a SPE cartridge filled with 30 mg molecularly imprinted polymers coupled with high performance liquid chromatographic detection. The MISPE/HPLC method was superior to the commercial C18 sorbents with regard to the matrix removal efficiency.

Keyword:
molecular imprinting, solid phase extraction, trichlorfon, spicy vegetables

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Introduction

Trichlorfon (O,O-Dimethyl-2,2,2-trichloro-1-hydroxy-ethyl-phosphonate ester) is an organophosphate insecticide used to control pests including cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leafminers and leaf-hoppers [1]. It is used widely to vegetables, fruits, field crops, livestock, ornamental and forestry plantings [2]. However, it is toxic by ingestion and dermal absorption, and has potential to cause tumors, genetic mutations, and reproductive effects [3-5]. Growing concerns over possible contamination and injury caused by trichlorfon has prompted the desire for the more sensitive and accurate methods for its analysis. Ordinarily, gas chromatography (GC) together with the matrix solid-phase dispersion (MSPD) has been used for the rapid screening of trichlorfon [6-8]. Besides considering the expensive instruments, the instability of trichlorfon at high temperature makes the GC analysis unreliable. Capillary Electrophoresis (CE) has also been used to detect trichlorfon due to its high efficiency and resolution, but it shows poor sensitivity and insufficient for direct determination of trace pesticide residues [9-10]. High performance liquid chromatography(HPLC) was developed based on catalytic effect of trichlorfon, since trichlorfon has the capacity to catalyze the oxidation of benzidine (4,4'-diamino-biphenyl) to 4-amino-4'-nitro biphenyl in the presence of sodium perborate, so the product of the catalyzed reaction was validated by LC-MS method [11-12]. Quantification of trichlorfon can also be achieved by monitoring the thermal breakdown products such as dichlorvos, dichloroacetaldehyde, and dimethyl phosphate [13-14].

Considering the analytical matrix effects by interfering compounds, an appropriate extraction process is required before the chromatography detection. Solid phase extraction (SPE) is widely used to purify the interest analytes from a wide variety of matrices. However, traditional SPE is challenged by complex sample matrices containing many interfering components [15-16]. Trichlorfon residue detection from spicy vegetables, such as ginger, allium fistulosum, onion, garlic and chili pepper has confronted with this challenge because of the abundant sulfhydryl compounds inside. When a kind of enzyme in these spicy vegetables exposed to the oxygen, they release a pungent organosulfur compound, which show the similar characters with the organophosphorus pesticides. Therefore, trichlorfon can hardly be isolated from the vegetable matrics with the commercially available solid-phase (C-18, alumina, silica-gel and polymer).

In recent years, one of the most interesting and promising methods for preparation of functionalized materials is the molecular imprinting technology (MIT). When template molecules were in contact with the polymer monomers to form multiple points, a memory imprint effect will be established through the aggregation process. After the template molecules removed, the polymer is formed with the template molecules in space configuration matching with the point of multiple hole having identify characteristics of template molecules and their analogues. The schematic of this technology was shown in figure 1.
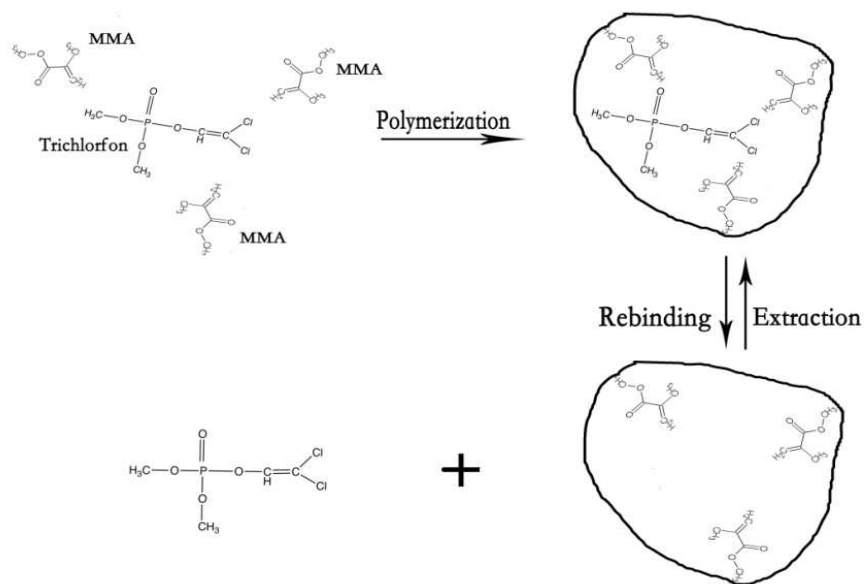
Fig.1 Schematic of molecular imprinting process

This method is reported as a synthetic approach to mimic natural molecular recognition elements [17-18], and the molecularly-imprinted polymers (MIPs) have been successfully utilized in SPE procedures for target analytes of biological and environmental samples [19].

In this work we reported an effort to isolate trichlorfon from complicated matrices using a MISPE method followed by HPLC detection. This procedure was carried out with several spiked spicy vegetable samples, and confirmed by the recovery and RSD. Compare with many techniques which have been reported for detect the trichlorfon, the MISPE method has the merits of low cost, good precision, low detection limit and wide linear range.

Experimental

1. 1 Chemicals

Trichlorfon was purchased from Dr. Ehrenstorfer Gmkh (Freiburg, German). Trimethylol propane trimethyl acrylate (TRIM), α- methacrylic acid（MAA） and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (Saint Louis, USA) and inhibitors were removed by an activated alumina column. The initiator 2,2'-Azobis-(2,4-dimethylvaleronitrile) (ABVN) was supplied by J&K Chemical Ltd (Beijing, China) and was recrystallized before usage. HPLC grade acetonitrile and methanol was purchased from Fisher (Waltham, USA). Other chemicals of analytical grade were supplied by Beijing Chemical Reagent Co (Beijing, China). All solutions were filtered through 0.45-μm membranes (Beijing, China) before usage.

1.2 Instruments

The detection of trichlorfon was performed on a Shimadzu HPLC system (Tokyo, Japan), which consists of two LC-20AT pumps, a SPD-20 UV detector and a SIL-10A auto sampler. A C18 column (250 × 4.6 mm, 5 μm) from Agela (Tianjin, China) was adopted. Acetonitrile/water (85/15,v/v) was used as mobile phase and the flow-rate was 1.0 mL/min. Aliquots of 5 μL of the reconstituted samples was injected for HPLC analysis, and detected at UV 200

nm. The porosity and surface area of the MIPs were characterized by the BET method using a Quadra Sorb SI 2000-11 (Quanta chrome, USA). The morphology of MIPs was obtained by a HITACHI S4800 field emission scanning electron microscope (Tokyo, Japan) with an accelerating voltage of 15 kV.

1.3 MIPs Preparation

Trichlorfon-imprinted polymers were prepared by dispersion polymerization method. Trichlorfon (0.1 mmol) and MAA (0.8 mmol) were dissolved into 35 mL acetonitrile, and stored for 12 h at 0°C, then TRIM (4.0 mmol) and ABVN (0.05 mmol) were added. Afterwards, the mixture was purged with nitrogen for 10 min, then polymerized in a UV incubator at 0°C for 24 hours. After the polymerization, the obtained polymers were firstly washed with methanol/acetic acid (80/20, v/v) for 8 hours four times on a transference shaker, and then washed with methanol 5~8 times until no trichlorfon can be detected in the rinses. The non-imprinted polymers (NIPs) were prepared in the same condition except in the absence of template.

1.4 Adsorption of trichlorfon on the MIPs/NIPs

Certain amount of MIPs/NIPs were suspended into methanol and diluted to 50 mg/mL. Each 100 μL MIPs/NIPs suspension was added into 900 μL trichlorfon standard solution in a series of known concentration which had been prepared in advance and filtrated through a 0.45μm filter from Millipore (MA, USA). The mixture was put into a shaker at 25 °C for 30 minutes followed by separation via centrifugation. The supernatant was analyzed by HPLC.

The adsorptivity at equilibrium was calculated according to the following equation:

$$Q = (C_0 - C_f) \ V/m \ .... (1)$$

Where C_0 (mmol L^{-1}) is the initial concentration of trichlorfon, C_f (mmol L^{-1}) is the concentration of trichlorfon in the supernatant at equilibrium, V (mL) is the volume of the adsorption mixture, and m (mg) is the mass of MIPs/NIPs. The dissociation constant K_d were determined by fitting the equation $y = B_{max} \times C_f / (K_d + C_f)$ (where y is the μmoles of target adsorbed per mg of polymer and C_f is the concentration of free target in μM, both at equilibrium) to the binding isotherm data at 25 °C using Prism 4 program from GraphPad Software Inc. The imprinting factor (IF) was defined as IF=$(B_{max(MIP)})/ (B_{max(NIP)})$. The detail for the batch-wise adsorption experiment was described as below.

1.5 Sample pre-treatment
First, the non-edible parts of ginger, allium fistulosum and onion samples were removed. Each sample (15 g) was taken and grounded in the juice machine. Then the samples were put into 50 mL centrifuge tube with 15 mL acetonitrile. Sample residues were gotten rid after 30 min ultrasonic extraction and centrifugal. Then 20 g active carbon was added in the vegetable juice and well mixed. After clarification, the mixture was filtrated by the air pump. Anhydrous magnesium sulfate (5 g) and sodium chloride (2 g) were following added. Samples were vortex oscillated for 2 minutes and centrifugal for 5 minutes at 3800 r/min. The supernatant liquid was taken out and filtrated through a 0.45 μm fliter.

1.6 Solid phase extraction

MIPs particles (30 mg) were packed into a SPE column. Prior to sample loading, the MISPE column was

pre-conditioned with 2.0 mL methanol and 2.0 mL ultra-pure water. Aliquots of 1.0 mL vegetable samples prepared in section 1.5 were eluted through the column at 0.1 mL/min. The washing procedure was performed with 1.0 mL methanol at a flow-rate of 0.1 mL/min. Finally, trichlorfon absorbed was washed away with 1.0 mL methanol/TFA(8/2, v/v), and the fractions were collected and evaporated to dryness using nitrogen. The residues were reconstituted in 1.0 mL acetonitrile/water (85/15, v/v) and then employed to HPLC analysis. For comparison, C18 SPE column with 30 mg C18 sorbents from Agela (Tianjin, China) was used to purify trichlorfon in the vegetable samples in the same condition as described above.

Results and Discussion

2.1 Morphology characterization

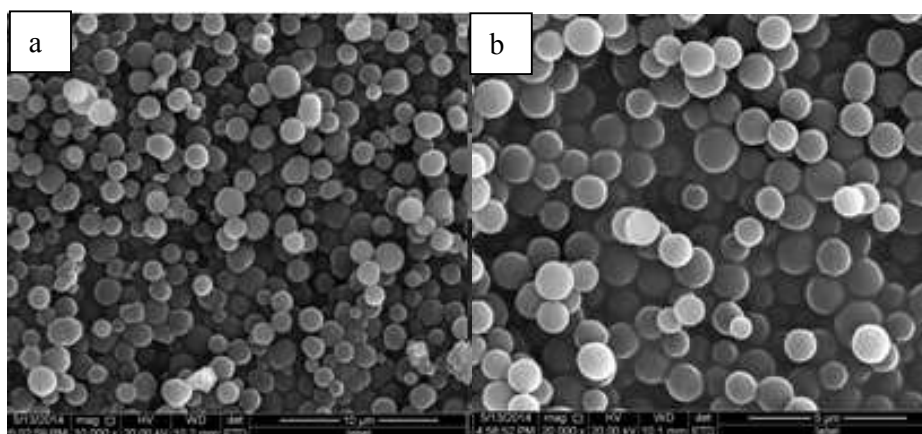Polymers prepared in this study were dispersed. The morphology of the MIPs/NIPs have been tested by SEM (figure 2).

Fig.2   SEM imagines of (a) MIPs and (b) NIPs

The SEM imagines of the polymer microspheres confirmed their spherical morphology with a narrow size distribution of 1–2 μm, and Figure 2a illustrated that the MIPs were porous. The surface of the MIPs was also characterized by BET experiment, the specific area is 7.62 m^2/g, the pore volume is 0.032 mL/g and the pore diameter is 17.05 nm.

2.2 Adsorption of trichlorfon on MIPs

MAA, TRIM and acetonitrile were used as monomer, cross-linker and solvent in preparing the MIPs respectively. The ratio between template, monomer and cross-linker is regarded as the key issue to control the affinity and selectivity for the MIPs, and some traditional ratios have been tested (Fig. 3).
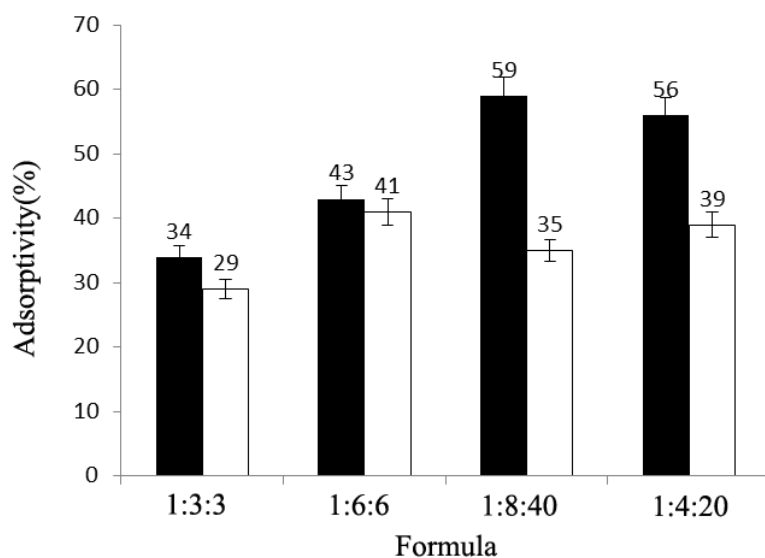
Figure.3 Effect of imprinting formula to the adsorptivity for the polymers

Imprinting formula represents the molar ratio between template, monomer and cross-linker.

The results show that when template, monomer, cross-linker with an imprinting formula of 1:8:40 was adopted, the MIPs show the best imprinting effect. When 10 μg/mL trichlorfon in methanol was incubated with 1.0 mg/mL polymers for 30 min, the adsorption of MIPs achieved to 59%, while the NIPs show only 35%. Variation of imprinting formula does not affect the affinity for NIPs obviously, but do affect the affinity for the MIPs significantly.

The binding isotherm is shown in Figure 4.The binding site capacity (Bmax) and dissociation constant K_d were determined by binding isotherm data at 25 °C. MIPs has a much higher Bmax (2.87 mmol/g) than that of NIPs (1.09 mmol/g), and a significant imprinting effect (IF= $Bmax_{(MIPs)}$/ $Bmax_{(NIPs)}$) of 2.64 was achieved. Meanwhile, the dissociation constant is 6.38 μM.
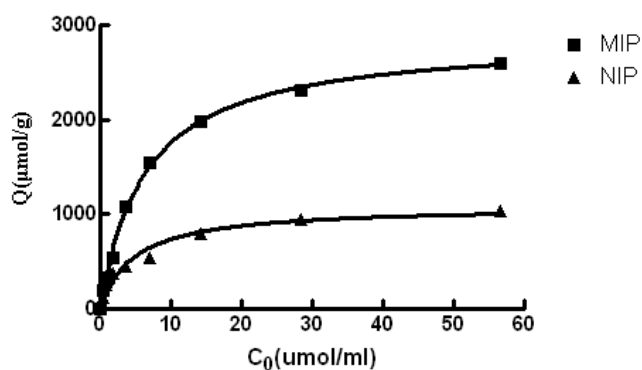

Figure 4 The binding isotherms of the polymers to trichlorfon

2.3 Extraction of trichlorfon from vegetables

Trichlorfon is a substance with a very short half-life. Therefore long term storage should be avoided and the analysis of trichlorfon by GC or HPLC after traditional SPE is difficult. An optimized extraction procedure by means of traditional SPE materials including C18 and polymers usually needs more than 400 mg solid-phase material [20], while 30 mg MISPE developed in this research can purify trichlorfon from ginger, allium fistulosum and onion samples efficiently. Here, vegetable samples spiked with trichlorfon were applied to the MISPE process. According to Table 1, recovery over 80% was achieved for samples spiked with 0.05 to 1 mg/kg trichlorfon, and the RSD for the established MISPE method is less than 3.5%.

Table 1   Recoveries of spicy vegetable samples spiked trichlorfon by MISPE/HPLC process (n=5)

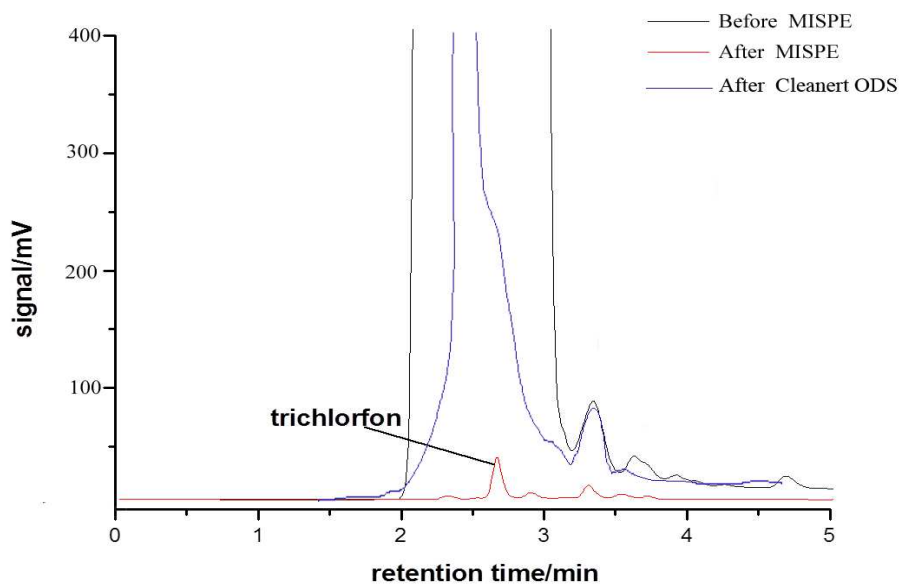| spiked concentration (mg/kg)	Ginger		Allium fistulosum		Onion	
	average recovery %	RSD%	average recovery %	RSD%	average recovery %	RSD%
1.0	85.4	3.1	80.2	3.3	82.5	2.7
0.5	82.9	2.9	84.1	2.5	83.3	2.9
0.05	89.3	3.5	80.6	3.2	82.5	3.4

Figure 5   HPLC analysis for trichlorfon purified from spiked ginger samples by MISPE and Cleanert ODS

Figure 5 shows a remarkable clean-up effect of MISPE to ginger samples spiked with trichlorfon. Since the crude extract from ginger contains numerous sulfhydryl compounds, the chromatogram of spiked ginger sample shows a big peak covering the peak of trichlorfon, and makes it impossible to both qualitatively and quantitatively determination. This was not improved with a traditional C18 SPE process which cannot purify trichlorfon selectively from ginger sample since sulfhydryl compounds and trichlorfon have similar adsorption effect on C18 adsorbent due to their similar polarity. But after MISPE process and washing procedure, trichlorfon can be detected by HPLC without obvious interference, which means no sulfhydryl compound is retained on the SPE cartridge.

This significant effect may come from the completely different space structure between sulfhydryl compounds and trichlorfon.

Besides ginger, MISPE can also be used for the purification of trichlorfon from allium fistulosum (Fig 6, A) , onion (Fig 6, B) successfully. Samples spiked with trichlorfon extracted from above vegetables can be purified by MISPE, and no significant interference can be observed on HPLC chromatograms. Moreover, a good linearity for HPLC analysis was achieved for ginger ($Y=3.2\times10^6x+2.4\times10^3$, $R^2=0.999$), allium fistulosum ($Y=1.3\times10^6x+1.0\times10^3$, $R^2=0.997$)and onion ($Y=4.2\times10^6x+2.0\times10^3$, $R^2=0.996$)samples spiked with 0.01 to 1 mg/kg trichlorfon. The detection limit of trichlorfon was 0.05 μg/mL

For other vegetables, including chili (Fig 6, C), garlic (Fig 6, D) and garlic shoot (Fig 6, E), although most interference was removed after MISPE process, the chromatographic separation is not good enough to discriminate trichlorfon clearly from interferences.
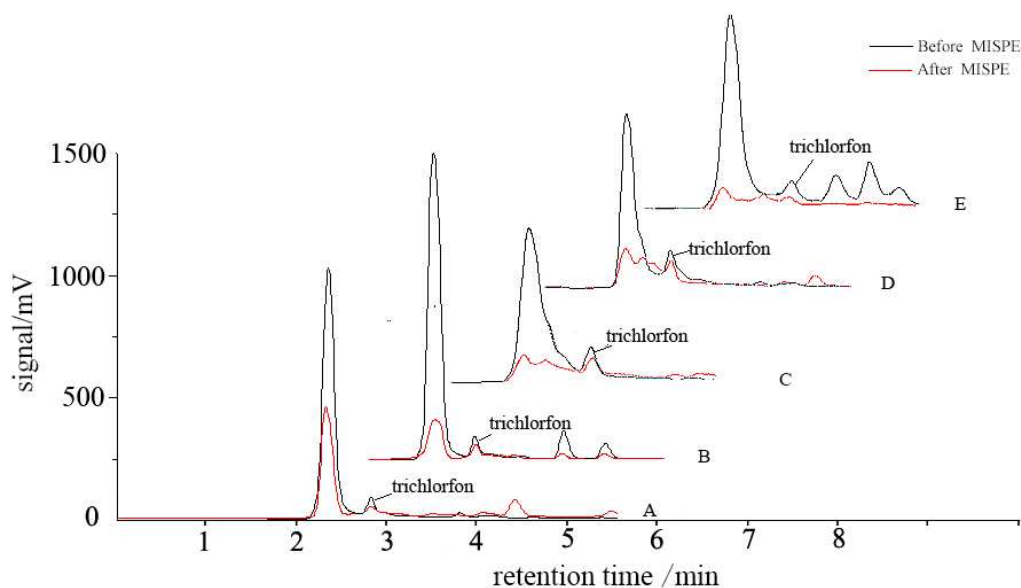

Figure 6. HPLC analysis for trichlorfon purified from spiked allium fistulosum samples(A), onion samples(B), chili samples(C), garlic samples (D), and garlic shoot samples (E) by MISPE.

Conclusions

It is essential to monitor the exposure levels and distribution of trichlorfon in vegetables, since trichlorfon is an organophosphorus pesticide which has toxicity effect and been ubiquitously found in vegetables. A rigorous challenge in determination of trichlorfon in vegetables is that their concentrations are usually ultra-low and its short half-life. This problem could be circumvented by using a simple MISPE strategy. Trichlorfon-imprinted polymers prepared by UV-initiated polymerization at 0°C exhibit a promising affinity and selectivity than NIPs. Relying on their good selectivity towards trichlorfon, MIPs were used as the sorbents of SPE columns. The recovery, precision and sensitivity of this MISPE-HPLC method were approved in spicy vegetable samples. The proposed method also provides an effective tool for monitoring the occurrence, distribution and fate of trichlorfon in the vegetables.

Acknowledgement

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