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Determination of dichlorvos by on-line microwave-assisted extraction coupled to headspace solid-phase microextraction and gas chromatography–electron-capture detection

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Abstract

The pretreatment technique of microwave-assisted extraction on-line headspace solid-phase microextraction (MAE–HS-SPME) was designed and studied for one-step in-situ sample preparation prior to the chromatographic analysis of a pesticide on vegetables. The pesticide on chopped vegetables was extracted into an aqueous solution with the aid of microwave irradiation and then directly onto the SPME fiber in headspace. After being collected on to the SPME fiber and desorbed in the GC injection port, the pesticide (dichlorvos) was analyzed with a GC–electron-capture detection system. The optimum conditions for obtaining extraction efficiency, such as the pH, the polarity modifier, and the salt added in sample solution, the microwave irradiation, as well as the desorption parameters were investigated. Experimental results indicated that the proposed MAE–HS-SPME technique attained the best extraction efficiency of 106% recovery under the optimized conditions, i.e. irradiation of extraction solution (10% aqueous ethylene glycol) at pH 5.0 with medium microwave power for 10 min. Desorption at 220 °C for 3 min offered the best detection result. The detection was linear at 5–75 μg/l with correlation coefficient of 0.9985. Detection limit was obtained at ~1.0 μg/l level based on $S/N=3$. The proposed method provided a very simple, fast, and solvent-less procedure to collect pesticides directly from vegetables for GC determination. Its application was illustrated by the analysis of trace dichlorvos in vegetables.

Keywords: Extraction methods; Headspace analysis; Vegetables; Food analysis; Dichlorvos; Pesticides

1. Introduction

The amount of pesticides routinely applied to agricultural commodities has dramatically increased in recent years. There are serious concerns about the increasing risks to human health. Thus, a rapid, efficient method to monitor pesticide residues in fresh fruits or vegetables is required because of the short time between harvest and sale in markets.

In conventional monitoring methods, large volumes of organic solvents are involved in several extraction and clean-up steps to remove the potential interfering species. This is hazardous to health and causes serious pollution problems in addition to the tedious and time-consuming procedures. Moreover, the disposal of solvent is very expensive. In order to increase the monitoring efficiency, previous studies have described various efforts at improvement such as fast GC [1,2], detection sensors [3–5], and pretreatment steps [6–15]. Among these, improve-

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ment in pretreatment has great potential to shorten the analytical time. Thus, many pretreatment protocols such as solid-phase extraction (SPE) [6,7], supercritical fluid extraction (SFE) [8,9], pressurized liquid extraction (PLE) [10,11], and microwave-assisted extraction (MAE) [7,12] have been examined. Although these methods offer efficient results, they are still relatively time-consuming. Therefore, solid-phase microextraction (SPME) was developed to resolve some of the problems [12–18]. However, the recovery of immersed SPME was found to be significantly influenced by sample matrix [19]. Therefore, headspace SPME (HS-SPME) was developed and applied successfully to avoid matrix effects [20–24]. However, HS sampling is only suitable for volatile species, as otherwise it takes a long time to finish the sampling and bad sensitivity and reproducibility are obtained. Thus, the headspace sampling technique is still limited to semi-volatile organic compounds.

Over the last decade, microwave-energy has been investigated and widely applied in analytical chemistry such as in accelerating sample digestion and extraction, and also in chemical reactions [25–27]. Through the dipole rotation and ionic conductance of polar substances or ionic species under microwave irradiation, the temperature rises very quickly. Therefore, microwave heating has the potential to improve HS-SPME sampling for organic compounds. Associating solid-phase microextraction with MAE, Wang et al. [28] first investigated the isolation of flavor ingredients in food products, Hernandez et al. [29] isolated herbicides in soil and water samples, and then Ho and Hsieh [12] isolated organo-chlorine pesticides from medicinal plants. They all were two-step procedures, i.e. the immersion of fiber into the aqueous solution followed by MAE. In addition, Zhu et al. [30] applied microwave mediated distillation with SPME to determine off-flavors in catfish tissue. The fiber was also immersed in the condensate to achieve the sample collection.

In this study, together with the advantages of SPME and MAE, microwave energy is proposed to assist one-step in situ extraction from vegetables and headspace sampling of pesticide on an SPME fiber. The MAE–HS-SPME technique coupled to GC–electron-capture detection is systematically investigated to develop a simple, fast, and solvent-less analytical process to determine pesticide residues in vegetables and fruits.

2. Experimental

2.1. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Barnstead, New York, USA) for all aqueous solutions. All chemicals and solvents were of ACS reagent grade. Dichlorvos was obtained from Riedel-de Häen (Seelze, Germany). Potassium dihydrogenphosphate and disodiumhydrogenphosphate were obtained from Merck (Darmstadt, Germany). The standard stock solution (100 μg/ml) of dichlorvos was prepared by dissolving 10 mg in 100 ml ethylene glycol (Riedel-de Häen) and stored in silanized brown glass bottles with PTFE-lined caps, and kept at 4 °C. Fresh working solutions were prepared by appropriate dilution of the stock solutions with ethylene glycol. Sodium chloride and sodium hydroxide were obtained from Riedel-de Häen. Hydrochloric acid (36.4%) was from J.T. Baker (Phillipsburg, USA). Citric acid was from TCI (Tokyo, Japan), and acetone was from Mallinckrodt (Paris, KY, USA). Vegetable and fruit samples were purchased from a local supermarket.

2.2. GC–ECD system

The GC system used was a Chrompack 9000 system (Middelburg, Netherlands) equipped with an ^{63}Ni electron-capture detector, and a splitter injector. Separations were conducted using a fused-silica DB-17 capillary column (30 m×0.32 mm I.D., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The temperature program used was as follows: 110 °C hold for 1 min, increasing temperature at 10 °C/min to 250 °C and hold for 2 min. The injector was used in the splitless mode and held isothermally at 220 °C for dichlorvos desorption (3 min). The ECD system was maintained at 250 °C. The carrier gas was nitrogen at a flow-rate of 1.0 ml/min, the flow-rate of the make-up gas was 18 ml/min with nitrogen, and the flow-rate of the purge gas (detector) was 15 ml/min. A Chem-Lab Data system (Chem-

Lab, Taipei, Taiwan) was used to obtain the chromatogram and perform data calculations.

2.3. MAE–HS-SPME system

The microwave oven used in the proposed system was modified from the home-used TMO-2030P system (2450 MHz, Tatung, Taipei, Taiwan) with a maximum power of 650 W, equipped with a cooling condenser connecting to tap water. After modification, microwave power was 11, 132, 160, and 210 W for weak, medium, medium high, and high irradiation, respectively. In order to keep the volume of headspace as small as possible, a glass tube was used to seal and guide the vapor through the SPME fiber. The MAE–HS-SPME system was set-up as Fig. 1.

The SPME device (Model 5-7330) consisting holder and fiber assembly for manual sampling was obtained from Supelco (Bellefonte, PA, USA) and used without modification. The fibers selected in the studies were 1-cm long coated with polydimethylsiloxane (PDMS) (100 μm film thickness). The fibers were conditioned under nitrogen in the hot injection port of the GC at 250 °C for 1 h prior to use. The needle on the SPME manual holder was set at its maximum length 4 cm in the GC injector port. A desorption temperature of 220 °C for 3 min was used to produce the highest sensitivity of dichlorvos. All analyses were performed with a 50-ml ground bottle containing 2 g of sample in 20 ml aqueous solution. In order to find the optimal microwave system and SPME parameters, 300 μg/l standard of dichlorvos was spiked into 20-ml aqueous solution.

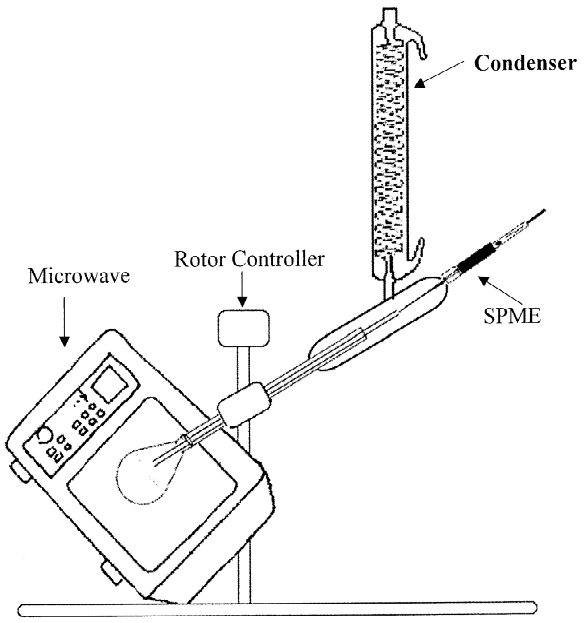

Fig. 1. Assembly of the MAE–HS-SPME apparatus.

3. Results and discussion

In order to optimize the MAE–HS-SPME sampling technique and the GC–ECD analytical conditions, factors affecting the sampling efficiency, such as the solution for MAE, the fiber, the microwave heating power and length of irradiation (fiber absorption time), the pH and the matrix modifiers in sample solution, as well as the desorption temperature and time, were studied thoroughly.

3.1. Selection of extraction solution

In the proposed method, the pesticide is extracted from samples (vegetable and fruit) under microwave irradiation and in-situ adsorbed onto SPME fiber located in the headspace. In this system, if a solvent were used to extract the pesticide, it would compete for the adsorption sites with the pesticide. Therefore, highly volatile solvents such as acetone and methanol (used in conventional methods) are unsuitable. Thus, the similarly polar ethylene glycol was tested as the modifier of extraction solution. Fig. 2 shows the influence of ethylene glycol in aqueous extraction solution on detection peak area (relative to extraction efficiency). It can be seen that 10% ethylene glycol content offered the best extraction efficiency to dichlorvos in vegetables.

3.2. Optimization of microwave irradiation conditions

In this study, MAE combined with HS-SPME is employed for directly collecting dichlorvos from vegetable. Parameters affecting heating including microwave irradiation power and irradiation time are investigated. Fig. 3 shows the recovery of dichlorvos on PDMS fiber during various irradiation times on

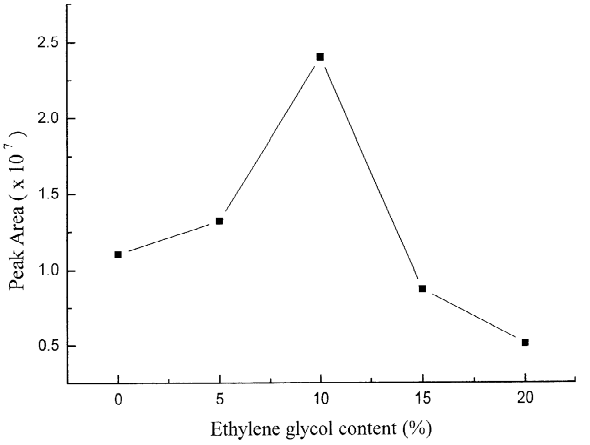

Fig. 2. Influence of ethylene glycol in aqueous solvent on extraction.

medium power (132 W). It can be seen that recovery increases with time and reaches an optimum at 10 min, and then decreases. This indicates that the dichlorvos might be lost due to its volatility and the adsorbed sites on fiber surface occupied by the vaporized ethylene glycol with longer microwave irradiation. In the series of studies for the recovery of dichlorvos under various microwave irradiation powers for 10 min, medium power (132 W) irradiation offers higher recovery compared with weak (11 W) and medium high (160 W) powers: irradiation under medium high power (160 W) and high power (210 W) would cause the loss of dichlorvos based on its volatility. Therefore, microwave irradiation with medium power for 10 min was recommended.

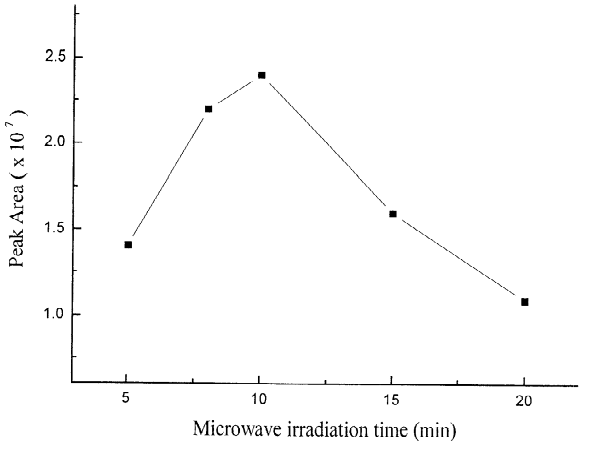

Fig. 3. Effect of microwave irradiation time on the recovery of dichlorvos. Microwave power: medium (132 W).

3.3. Fiber selection and microwave-assisted headspace solid-phase microextraction

As dichlorvos is a partial polar species, the PDMS fiber was selected for the extraction of dichlorvos. The proposed HS-SPME extraction is a dynamic partition process among the SPME fiber, headspace, extraction solution, and sample. Because the HS-SPME sampling system is set on-line to microwave, in addition to the fast extraction of dichlorvos into extraction solution, the quickly rising temperature increases the vapor pressure of dichlorvos favoring the absorption of dichlorvos on the fiber. As described in Fig. 3, 10-min exposure of the fiber on headspace obtained maximum quantity of dichlorvos under medium microwave irradiation power. Therefore, the fiber was withdrawn from the sampling assembly after a 10-min exposure in the headspace during microwave irradiation.

3.4. Thermal desorption temperature and desorption time

For better separation efficiency and resolution, thermal desorption requires a possible minimum time. Dichlorvos has boiling point of 170 °C and is stable at high temperature. After a series of tests, as in other SPME analytical studies, the detection peak area of dichlorvos increased with desorption temperature and desorption time, but there was no significant increase after 220 °C for 3-min desorption. With these conditions, no significant blank value was observed. Thus, no further regeneration mode for the fiber was necessary.

3.5. Influence of the pH of extraction solution on extraction

The pH of the sample solution is often adjusted to enhance the extraction efficiency in conventional liquid–liquid extraction, solid-phase extraction, and solid-phase microextraction. Fig. 4 shows the effect of pH in sample matrix on the extraction efficiency (detection peak area) of dichlorvos. It can be seen

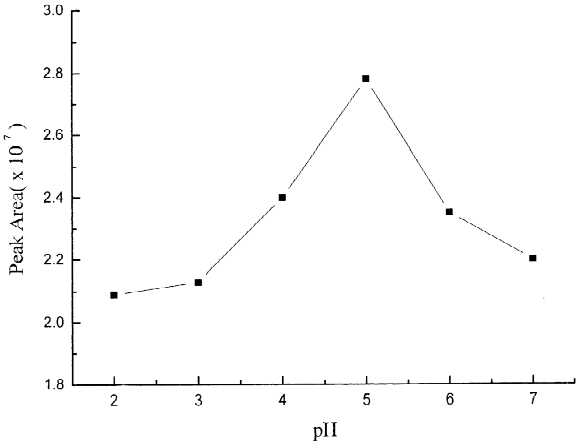

Fig. 4. Effect of pH in extraction solution on extraction efficiency.

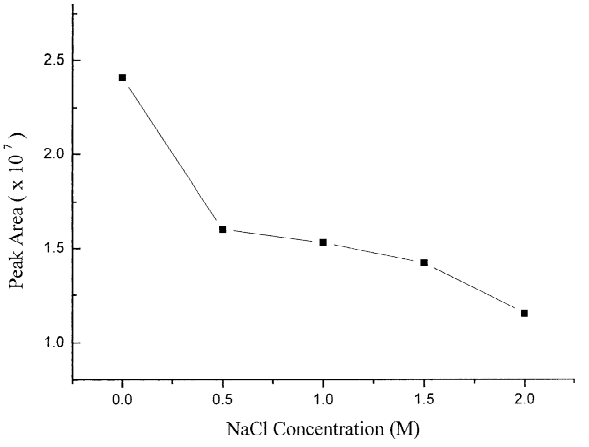

Fig. 5. Effect of NaCl in extraction solvent on extraction efficiency.

that the detection peak area increases above pH 3.0, is optimum at pH 5.0, and then decreases. Because dichlorvos is not significantly ionized in aqueous solution due to its pK_a value, it might hydrolyze in extreme pH aqueous solution. Therefore, the extraction solution was adjusted to pH 5.0 to obtain the best extraction efficiency.

3.6. Effect of salt addition

A salt-out effect often improves recovery in conventional extraction processes. In the direct immersed SPME, addition of ionic salts to sample solution could decrease the solubility of analyte, and enhance the extracted quantity [23,31]. Thus, NaCl or Na_2SO_4 is usually added into the aqueous sample. However, some discrepancies have been found and no direct relation between extraction efficiency and salt addition has been pointed out [32–36]. In this study, NaCl of different concentrations (from 0 to 2.0 *M*) was added to aqueous extraction solution to observe its effect on extraction. Fig. 5 shows the effect of NaCl in extraction solution on the extraction efficiency. It can be seen that the extraction efficiency decreases with NaCl addition. This indicates that the dichlorvos is liable to salt-out from aqueous solvent onto the sample particles, which decreases the extraction efficiency. Therefore, salts were not added into extraction solution in the MAE–HS-SPME system.

3.7. Validation of the method

In order to test the applicability of the proposed MAE–HS-SPME–GC–ECD method for quantitative determination of dichlorvos, standard solutions (dichlorvos spiked in 20 ml 10% ethylene glycol aqueous solution) were used for calibration after they were subjected to the overall treatment procedure, i.e. MAE–HS-SPME and thermal desorption from the fiber into the chromatographic system. An ECD chromatogram of dichlorvos standard under the chromatographic conditions described in the Experimental section is shown in Fig. 6. Calibration plots were built up over the concentration ranges of 5–75 μg/l, with equation $Y = 76\,420X - 148\,500$. The linear relationship between the peak area and the spiked quantity of dichlorvos was in good agreement with the correlation coefficient of 0.9985. The detection limit was calculated based on three times the average background noise divided by the detection sensitivity (slope of calibration plot), which was 1.0 μg/l. The precision of this method was estimated by performing five extractions of pH 5.0 extraction solutions spiked with dichlorvos at concentrations for calibration plot. The precisions ranged between 5.5 and 7.9% RSD.

For examining the applicability of the method to determine dichlorvos in real samples, tomato, strawberry, and pakchoi obtained from a local supermarket were analyzed. Results indicated that residual

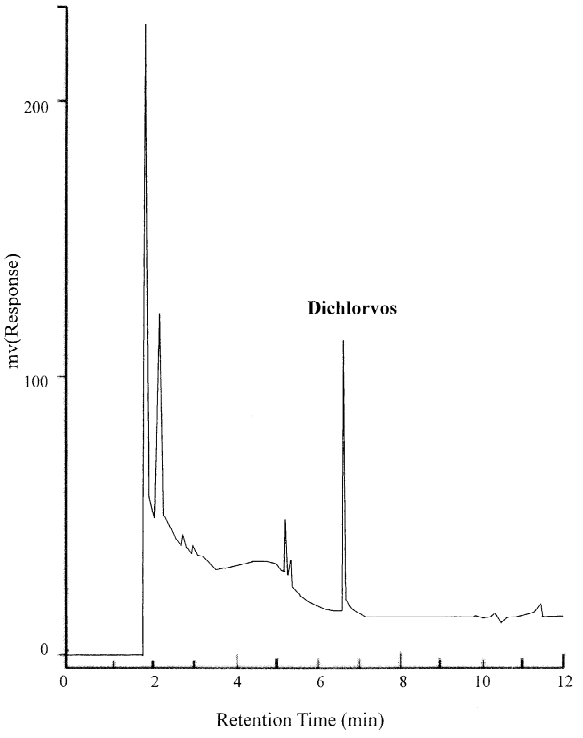

Fig. 6. Chromatogram of dichlorvos standard solution with the proposed method. Concentration: 300 μg/l.

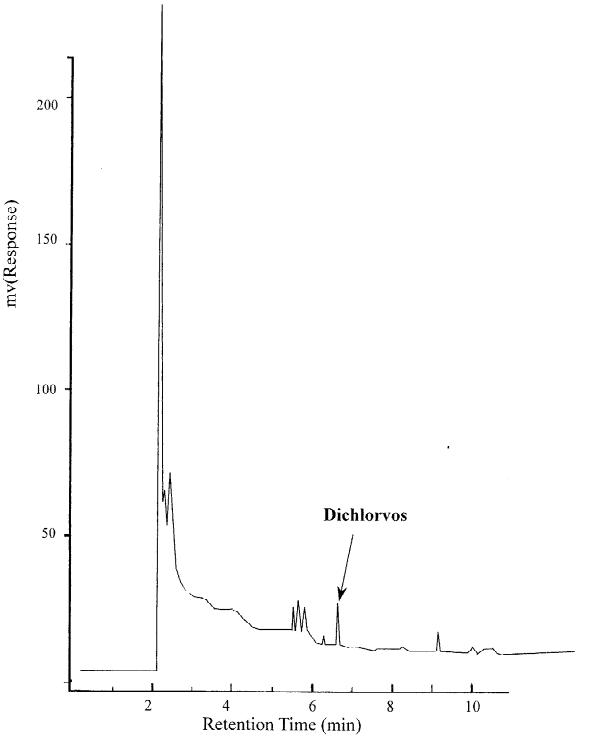

Fig. 7. Chromatogram of dichlorvos in the sample of pakchoi. Concentration: 8.65 μg/l.

dichlorvos (8.65 μg/l) was only found in the pakchoi with none in the other two samples. Fig. 7 is the chromatogram of dichlorvos in the pakchoi sample. When the pakchoi sample is spiked with 10 μg/l dichlorvos, recovery is 106.1% after MAE–HS-SPME extraction–thermal desorption–GC–ECD determination with optimal conditions. The accuracy and precision are acceptable in the residual analysis of pesticide in vegetables. Compared to conventional analytical methods, the time for sample pretreatment in the proposed method is reduced to 10 min only, and no toxic organic solvents are required. It is a fast and environmentally acceptable method.

3.8. Comparison with SPME and MAE

The SPME was developed to solve the problems caused by the use of toxic organic solvents in sample pretreatment. Although immersed SPME is solventless, it usually takes 30–60 min to achieve a sampling and suffers from the matrix effect. As regards HS-SPME, it is free of matrix effect, but it is limited to collecting volatile species, as it takes several hours to collect less volatile species. The application of MAE is to obtain a higher level of extraction efficiency within a short time from a sample matrix. However, organic solvent is still required and further pretreatment is required prior to chromatographic determination. As described previously, the MAE–HS-SPME system is proposed having the advantages of both MAE and HS-SPME. It takes only 5–15 min to complete a sample pretreatment for semi-volatile sample species without the use of toxic organic solvents.

4. Conclusion

In this paper, determination of a residual pesticide (dichlorvos) by the proposed MAE–HS-SPME–GC–ECD system has been described, and the optimal conditions have been established. From the results,

the proposed method has been proved capable of analyzing a pesticide (dichlorvos) in vegetable samples with the advantages of being fast, convenient, and free from toxic organic solvents.

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