Using Fast Gas Chromatography–Mass Spectrometry with Auto-Headspace Solid-Phase Microextraction to Determine Ultra Trace Residues of Organophosphorus Pesticides in Fruits

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Abstract

Organophosphorus pesticides (OPs) in apple and tomato were determined by using fast gas chromatography–mass spectrometry (FGC-MS) coupled with auto-headspace solid-phase microextraction (SPME). The experimental conditions of FGC were investigated and developed. Three different fibers were studied and compared and it was found that the polydimethysiloxane (PDMS) was the best. Factors affecting the extract efficiency, such as extraction time, temperature, ion strength, agitator speed, and content of organic solvents, were investigated and optimized. The limits of detection (LOD) of OPs were achieved between 0.002 ng/g and 0.955 ng/g; the RSD was less than 20.9%, and the recoveries were from 79% to 117%. For most commercial fruit samples, ultra trace residues of OPs were found, mainly on the surface, and their concentrations were generally lower than LOD of conventional methods.

Introduction

The organophosphorus pesticides (OPs) have been widely used in agriculture because of their ability to degrade more readily in the environment. However, it may lead to the occurrence of residues in food commodities, water, and soil. Ingesting OPs from contaminated foods can disrupt nerve function by binding to the enzyme acetylcholinesterase, which will result in paralysis and death (1). Thus, the contamination of OPs in foods and environment has become an extensive concern in the world. It is necessary to develop some fast and sensitive methods to determine ultra trace residues of OPs.

Gas chromatography (GC) coupled with different detectors is the most commonly used analytical method for determination of OPs, combined with traditional sample preparation techniques such as solid-phase extraction (SPE) and liquid–liquid extraction (LLE) (2). The conventional analytical techniques generally include the following process: sampling, extraction, concentration, and detection. These steps are time consuming, expensive, and have to be performed with toxic reagents. Thus automated sample preparation methods have become particularly attractive. Solid-phase microextraction (SPME), introduced first in 1990 by Arthur and Pawlizyn (3), integrates sampling, extraction, concentration, and sample introduction to GC. It offers high through-put performance without extending sample preparations. SPME is reproducible, inexpensive, simple, and effective in that it eliminates much interference from the sample matrix, and thus the selectivity is improved. In addition, SPME can be coupled with GC techniques easily and be automated by the SPME auto-sampler system.

Since 1992, a number of SPME methods have been developed to extract flavors, off-flavors, pesticides, and other contaminants from various food samples, such as vegetables, fruits, beverages, dairy products, and meats (4). SPME technique for determination of pesticides in food samples had been reviewed (5,6). However, fewer SPME methods have been used in the analysis of pesticide residues in agricultural commodities because the complex matrices of such products may cause interference in the extraction procedure (7). Up to now, a few authors have described the viability of SPME as an extraction technique in complex matrices, such as fruits (7,8), fruit juice (7,9), vegetables (10), bee honey (11), wines (9), and plants (12). The main drawback of SPME was that it was primarily a manual system, which is affected by the experiment conditions with poor reproduction compared with auto SPME. Recently, several companies have introduced SPME capable autosampler systems. This type of instrument, completely automating the SPME process with only minimum sample handling by the analyst, becomes ideal for working with toxic analytes. It had been successfully used in the analysis of tetramethylene disulfotetramine in foods (13).

Fast gas chromatography (FGC) allows faster analysis than conventional capillary gas chromatography (14–18). FGC adopts shorter and narrower capillary columns with the thinner film of the stationary phase. The speed of analysis is improved by increasing the temperature-heating rates and applying a detector, such as mass spectrometer at a lower outlet pressure. FGC separates component peaks rapidly while the mass spec-

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trometry identifies the unresolved peaks well. Integrated with these two apparatus, analysis can meet the demand to shorten the analytical time and improve the analytical efficiency in detecting multiple OP residues in foods. The peak is sharper and higher acquired by FGC than by conventional GC. In other words, FGC achieves a higher signal-to-noise (S/N) ratio while the sensitivity is increased.

In this paper, a novel technology was developed by integrating auto SPME, FGC and mass spectrometry to determine ultra trace resides of multi OPs in fruits. It was successfully applied to determine ultra trace residues of OPs in fruits.

Experimental

Reagents

All regents were analytical grade or higher. Solvents were of HPLC grade and obtained from Merck (Darmstadt, Germany). OPs standards (phorate, iprobenfos, methyl parathion, chlorpyrifos, fenitrothion, malathion, fenthion, ronnel, parathion, bromophos methyl, quinalphos, phenthoate, ethion, and carbophenothion) were purchased from the Chinese National Standard Material Center (Beijing, China). Concentration of each OPs (dissolved in acetone) was 100 µg/mL. Cyanophenphos was obtained from Dr. Ehrenstorfer (Augburg, Germany) with 99% purity. An organophosphorus (1 µg/mL) mixed standard solution was prepared by dissolving an appropriate amount of above individual standard compound in acetonitrile and was stored in the dark at 4°C. Standard solutions were diluted for practice use.

Instrumentation

GC–MS analysis was performed by GCMS-QP2010 gas chromatography mass spectrometer (Shimadzu, Kyoto, Japan), equipped with a split/splitless injector and a Shimadzu auto injector AOC-5000 (CTC, Switzerland) controlled by Cycle Composer software (CTC, Switzerland). Data were acquired through GCMS solution software (Shimadzu).

The GC column consisted of a fused silica Rtx-5 capillary column (10 m × 0.10 mm i.d.) with a 0.10-µm film thickness (Restek, Bellefonte, PA), connected to the split/splitless injector. The i.d. of injector liner was 0.75 mm. Helium was adopted as the carrier gas. The same apparatus was applied for traditional GC analysis except to use Restek fused silica Rtx-5Sil MS capillary column (30 m × 0.25 mm i.d.) with a 0.25-µm film thickness (Restek).

Chromatographic and detection conditions

Two capillary columns and three chromatographic conditions were used in this work. Condition A used a microbore capillary column (i.d. 0.10 mm). The column head pressure was 550 kPa, and the temperature program was as follows: from 40°C (1 min) to 130°C at 120°C/min; from 130°C to 250°C at 60°C/min; from 250°C to 300°C at 120°C/min (1 min). The injector was operated in splitless mode for 1 min (sampling time). Condition B used a microbore capillary column (i.d. 0.10 mm). The column head pressure was 389 kPa, and the temperature program was as follows: from 40°C (1 min) to 130°C at 128°C/min; from 130°C to 250°C at 21°C/min; from 250°C to 300°C (1 min) at 150°C/min. The injector was operated in splitless mode for 1 min (sampling time). Condition C used a narrow bore capillary column (i.d. 0.25 mm). The column head pressure was 63.9 kPa, and the temperature program was as follows: from 40°C (1 min) to 130°C at 30°C/min; from 130°C to 250°C at 5°C/min; from 250°C to 300°C at 10°C /min (5 min). The injector was operated in splitless mode for 1.5 min (sampling time).

The injector temperature was set at 250°C, whereas the GC transfer line was maintained at 280°C. Then the mass spectrometer was operated in the electron impact positive ion (EI+) mode with a source temperature of 230°C.

In order to improve peak identification, chromatograms were acquired in selected ion monitoring (SIM) mode. Fragment ions were monitored for each analyte as specified in the following: *m/z* **260**, 231, event time 0.10 s (phorate); *m/z* **204**, 246, 288, event time 0.12 s (iprobenfos); *m/z* **263**, 233, 246, event time 0.12 s (methyl parathion); *m/z* **285**, 287, 125, Event time 0.12 s (fenchlorphos); *m/z* **277**, 260, 247, event time 0.18 s (fenitrothion); *m/z* **173**, 158, 143, Event time 0.18 s (malathion); *m/z* **278**, 169, 153, event time 0.18 s (fenthion); *m/z* **314**, 258, 286, event time 0.18s (chlorpyrifos); *m/z* **291**, 263, 186, event time 0.18 s (parathion); *m/z* **331**, 329, 213, event time 0.18s (bromophos methyl); *m/z* **146**, 298, 157, event time 0.10 s (Quinalphos); *m/z* **274**, 246, 320, event time 0.10s (Phenthoate); *m/z* **231**, 199, 384, event time 0.10s (ethion); *m/z* **157**, 199, 342, event time 0.10s (Carbophenothion); *m/z* **157**, 169, 141, event time 0.10 s (Cyanophenphos). The quantification ion for each analyte was boldfaced *m/z*.

Sample preparation

Fruit samples (obtained from local markets and were stored at 4°C) were treated as follows: a 20.0 g of sample was homogenized along with 40 mL of acetonitrile and 5 g of NaCl by using a high-speed blender for 1 min. The resultant solutions were centrifuged at 3000 rpm/min for 5 min. Then 0.75 mL of 50 mL suspension diluted by acetonitrile was transferred into a 20 mL vial (Alltech, Deerfield, IL). NaCl (4.5 g) was added and diluted to 15 mL with water. The vial was sealed with 20 mm silicone/PTEE magnetic crimp-top cap (CNW Technologles GmbH, Germany).

HS-SPME analysis

Three types of fibers (Supelco, Bellefonte, PA), 100-µm PDMS (polydimethylsiloxane), 85-µm PA (polyacrylate), and 50/30-µm DVB–CAR–PDMS (divinylbenzene–carboxen–polydimethylsiloxane), were used in the experiments. All determinations were based on a 100-µm PDMS fiber which was mounted in a SPME adapter of the auto injector. The process of HS-SPME was performed automatically under the control of Cycle Composer software. The sample vial transferred to the agitator was preheated at 70°C with 750 rpm/min for 1.0 min. After being exposed in the headspace of sample vial for 80 min, the fiber was inserted into the injector of the GC for desorption (~ 6 min). Then the HS-SPME process began running another sample. The fiber was baked automatically for 5 min at 260°C in a separate "bake-out" station with gas N_2 every 5 HS-SPME runs to keep desorbing completely. All fibers were initially conditioned according to the

manufacturer's instruction. PDMS fiber was conditioned at 250°C for 30 min, PA fiber at 280°C for 60 min, DVB-CAR-PDMS fiber at 270°C for 60 min. A fiber blank run was performed at the beginning of each working day to prevent from contaminating.

Quantification

Quantification was performed by using spiked samples. The homogenized blank fruit matrix was obtained as described in Sample preparation section. 360 µL of mixed standard solution (containing 1.0 µg/mL of each OP except the concentration of chlorpyrifos is 0.5 µg/mL) in acetonitrile was mixed with 1640 µL of blank fruit matrix. Then 0.75 mL of mixed solvent was transferred into the headspace vial. NaCl (4.5 g) was added and diluted to 15 mL with water. The amount of each OP is 120 ng (the amount of chlorpyrifos is 60 ng) in the headspace vial. That is to say, the amount of each OP is 8000 ng (the amount of chlorpyrifos is 4000 ng) in 50 mL of fruit matrix. Therefore, the final concentration of OPs of resulting spiked sample is 400 ng/g (concentration of chlorpyrifos is 200 ng/g). One milliliter of the described mixed standard solution was double diluted with blank fruit matrix. A series of spiked blank fruit matrixes were prepared using the same procedure, and were used in the linearity studies.

Results and Discussion

Fast gas chromatography (FGC)

There are some factors that affect separation speed and resolution of FGC, such as the length and the diameter of the column, the thickness of film, carrier gas, and temperature programming (16). In this paper, a microbore capillary column (i.d. 0.10 mm) was used for FGC, compared with a narrow bore capillary column (i.d. 0.25 mm) for conventional GC. Both columns were made by the same company, which has the same stationary phase but different diameter, length and film thickness. 0.1 µg/mL (for each) of mixed OPs standard solution was injected into GC directly to compare fast GC to conventional GC.

Figure 1 shows the chromatogram separation of mixed OPs under three different chromatographic conditions. Figure 1C is a conventional technology using a narrow bore capillary column (i.d. 0.25 mm). Chromatographic condition was set as national standard of China for determination of 446 pesticides residues in fruits and vegetables (19). The temperature program in Figure 1B can be translated from the condition in Figure 1C by GC method translation software (20). The analytical time is shortened from 38.0 to 8.75 min. While column head pressure was increased to 550 kPa. Thus, the analytical time was shortened further to 5.17 min as seen in Figure 1A. Table I presents the eluted temperature of OPs under three different chromatographic conditions. We can see that the eluted temperature of

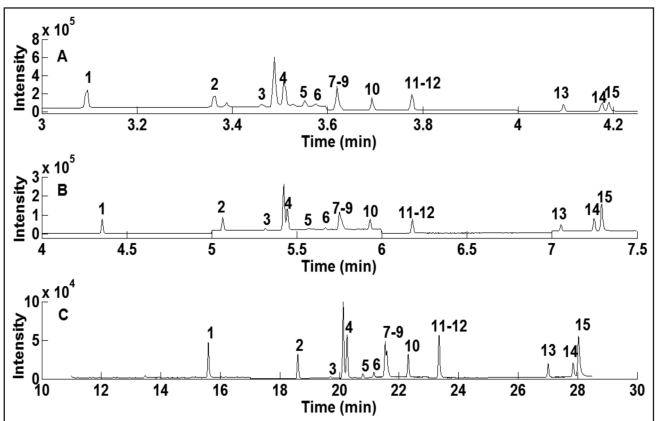

Figure 1. GC–MS (SIM) chromatograms of OPs standards at 0.1 µg/mL with column head pressure of (A) 550 kPa; (B) 389 kPa; (C) 63.9 kPa. Phorate, 1; iprobenfos, 2; methyl parathion, 3; ronnel, 4; fenitrothion, 5; malathion, 6; fenthion, 7; chlorpyrifos, 8; parathion, 9; bromophos methyl, 10; quinalphos, 11; phenthoate, 12; ethion, 13; carbophenothion, 14; cyanophenphos, 15.

Table I. Eluated Situation for OPs Under Different Chromatographic Conditions

	0.10 mm column, 550 kPa				0.10 mm column, 389 kPa				0.25 mm column, 63.9 kPa			
	RT* (min)	h†	$w_{1/2}$‡ (s)	ET§ (°C)	RT* (min)	h†	$w_{1/2}$‡ (s)	ET§ (°C)	RT* (min)	h†	$w_{1/2}$‡ (s)	ET§ (°C)
Phorate	3.096	35058	0.7	210.8	4.362	19020	1.1	185.7	15.633	6986	5.1	188.0
Iprobenfos	3.367	71837	0.8	227.0	5.071	43022	1.2	200.5	18.638	17087	5.6	203.0
Methyl parathion	3.467	6061	0.6	233.0	5.328	3250	1.1	205.9	19.739	515	7.1	208.7
Ronnel	3.514	101050	0.7	235.8	5.458	46562	1.2	208.6	20.292	20507	5.3	211.3
Fenitrothion	3.551	4904	0.7	238.2	5.584	3187	1.2	211.2	20.834	705	6.1	213.9
Malathion	3.580	6769	0.8	239.8	5.682	5116	1.2	213.3	21.210	891	5.1	215.8
Fenthion	3.623	159490	0.6	242.4	5.768	56442	1.3	215.0	21.569	19572	5.6	217.7
Chlorpyrifos	3.629	41823	0.6	242.8	5.78	13548	1.2	215.3	21.627	5106	4.6	218.0
Parathion	3.633	11779	0.7	243.0	5.792	6344	1.1	215.5	21.677	2808	6.1	218.2
Bromophos	3.700	78228	0.5	247.0	5.947	28854	1.2	218.8	22.347	6672	5.4	221.5
Quinalphos	3.783	65902	0.7	254.0	6.196	28984	1.4	224.0	23.340	6930	5.6	226.7
Phenthoate	3.783	35203	0.6	254.0	6.200	15091	1.0	224.0	23.392	3694	4.7	226.8
Ethion	4.099	51522	0.6	291.9	7.074	22193	1.1	243.3	27.058	8352	5.4	245.1
Carbophenothion	4.180	52587	0.6	300.0	7.270	46725	1.2	246.4	27.903	5489	7.0	249.3
Cyanophenphos	4.190	107264	0.7	300.0	7.315	92007	1.4	247.4	28.104	6246	9.6	250.4

* Retention time; † Height of peak; ‡ Width of half height of peak; § Eluated temperature.

OPs being obtained under condition C is much different from condition A or B. Therefore, GC method translation software is efficient on translating standard GC method into FGC ideally without re-adjusting chromatographic condition.

Figure 1 and Table I show that the analytic time is shortened after FGC adoption. Moreover, the response of peak is enhanced while the noise is not increased accordingly. It is interesting to see from Table I that height of peak is inversely proportional to width at half height of peak, although they are not exactly matched mathematically. The height of peak being obtained by FGC is significantly higher than that by traditional GC. The largest response was obtained by method C with 550 kPa of column head pressure. There are only 3–4 points of sampling data for each peak with method C due to the limitation of the sampling time of the mass spectrometer detector used in this study. Normally at least 8 points of each peak is necessary for good estimation (16). If 550 kPa or above of column head pressure was adopted, the higher signal-to-noise ratio can be achieved in screening of ultra trace of OP residues. Therefore, condition B was chosen in this paper because it can meet the demand of qualification with much better S/N than condition A.

Influence of matrix

According to the literature (21), 5 g of fruit was homogenized along with 5.0 mL of water by using a high-speed blender, and 6.0 mL of the fruit homogenate was transferred to the headspace vial before 1.6 g of NaCl added. The mixed OPs standard solution was spiked in homogenate. Due to the complicated matrix in the fruit, the competition between compounds and analytes of the matrix adsorbed by SPME fiber results in lower extraction efficiency. Only parathion, methyl parathion, fenitrothion, and malathion described in literature can be extracted. In order to reduce the matrix effect, 20 g of sample was extracted with 40 mL acetonitrile, and then 0.75 mL of 50 mL suspension diluted by acetonitrile was diluted to 5% with water in the headspace vial. The contents of the solution after extraction were relatively simple in thhe headspace vial. Extraction of OPs was in linear range of fiber through reducing matrix interference.

Optimization of the SPME

In order to optimize the SPME conditions, the mixed OPs standard solution was added in the headspace vial. The final content of individual OPs is 30 ng. 750 µL of acetonitrile and 3.0 g of NaCl were added in the vial and diluted to 15 mL with deionized water. Optimization of SPME was achieved according to suggested condition.

Selection of extraction mode

There are two sampling modes for SPME: direction insertion (DI) mode and headspace (HS) mode. They were compared by analyzing the same samples using PDMS fiber under the same extraction condition. Extraction temperature is 40°C, extraction time is 60 min, and agitate speed is 500 rpm. Response for DI is a little higher than that for HS. However, the lifetime of fiber in the DI sampling mode may be shortened by the addition of salts and coexisting compounds of the complex matrix in real sample. Finally, HS-SPME sampling mode was chosen in this work.

Selection of fiber coatings and optimization of extraction time

In the early stages of SPME, the amount of analytes absorbed by the fiber increases rapidly. The absorbtion speed becomes slow near to the equilibrium point. Because there exists a linear relationship between the amount of analyte absorbed by the SPME fiber and its initial concentration in the sample matrix is under non-equilibrium conditions (4), full equilibration is not necessary for precise quantification by SPME.

Extraction efficiency of PDMS, PA, and DVB–CAR–PDMS to OPs varies according to the principle of "like dissolves like". The three fibers are non-polar, more-polar, and mixed coating fiber, respectively. The relation between extraction time and extraction efficiency was studied basing on the three fibers at extraction temperature of 40°C and agitation speed of 500 rpm. The extraction efficiency of OPs is increased along with increasing extraction time. Although the extraction modes of the three fibers are identical, it is obviously seen that extraction efficiency with DVB–CAR–PDMS fiber is the lowest. Thus, DVB–CAR–PDMS is not discussed in subsequent analyses. Extraction efficiency with PDMS is a little better than with PA when extraction time is 100 min. Because equilibrium was reached for parathion, phenthoate, fenitrothion, iprobenfos, quinalphos, methyl parathion, malathion, and cyanophenphos with PDMS after 80 min, whereas equilibrium with PA was not reached. From 80 to 100 min, response for most analytes with PA increases rapidly and tends to increase dramatically afterwards. The similar result was found by other people (11), and the response for OPs continued to increase until 160 min.

PDMS fiber has the property of liquid whereas PA fiber is solid. Although PDMS film is thicker, it has faster diffusion and reaches equilibrium within a shorter time (22). Moreover, the stability of a PDMS coat is favored by automated SPME. Equilibrium of OPs extracted with PDMS was reached at ~ 80 min, where extraction efficiency of most analytes is almost as high as that with PA at 100 min. A shorter extraction time could shorten analytical time and prolong the life of fiber. Therefore, PDMS fiber and extraction time of 80 min were chosen for subsequent analyses in this work. It was found that extraction efficiency of phorate, chlorpyrifos, fenchlorphos, and bromophos methyl was between 23 and 60%, compared to extraction efficiency of other OPs between 0.23% and 3.2%. Figure 2 compares extraction efficiency of three fibers when extraction time is 80 min; it is found that the

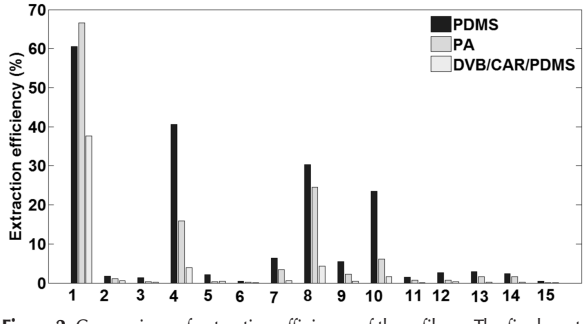

Figure 2. Comparison of extraction efficiency of three fibers. The final content of individual OPs in the vial is 30 ng. 750 µL of acetonitrile and 3.0 g of NaCl were added in vial and diluted to 15 mL with deionized water. Extraction temperature is 40°C, extraction time is 80 min and agitation speed is 500 rpm.

extraction efficiencies of OPs with PDMS and PA fiber are evidently greater than that with a DVB–CAR–PDMS fiber. Compared with the PA fiber, PDMS fiber presented more efficiency, except phorate, and the efficiencies for most OPs with PDMS fiber are more than 50% that with PA fiber.

Selection of extraction temperature

With increasing extraction temperature, the molecule will get much energy. It enhances the mass transfer process, increases the vapour pressure of the analytes (23), and facilitates the release of analytes into headspace (24). However, the adsorption of the volatile by the fiber coating is an exothermic process, and the high temperature is good for the release of analytes from their matrix, but it can adversely affect the adsorption of analytes.

Temperature effect was evaluated from 35°C to 85°C for SPME. The amount of each analyte extracted by PDMS fiber was plotted against the extraction temperature (see Figure 3). It can be seen that only the response of phorate decreases with increasing temperature and finally disappears at 80°C. The reason might be that phorate is adsorbed by fiber much more easily at lower temperatures, and desortion becomes the main action when temperature rises. Response of chlorpyrifos, fenchlorphos, and bromophos methyl, whose extraction efficiencies are much better, reaches maximum at 60°C, whereas others' responses reach the highest point at 70°C. Thus, the optimum extraction temperature was selected to be 70°C in this work.

Selection of agitation speed

More reproducible results can be obtained through maintaining a constant agitation speed during extraction with automated SPME. The range of agitation speed is 100–750 rpm, and the effect of agitation speed was evaluated by adopting agitation speed of no agitation, 250, 500, and 750 rpm. Extraction efficiency of all OPs, except phorate, increases along with agitation speed. Response of phorate decreased until agitation speed increased at 500 rpm, and then its response increased rapidly. When agitation speed was 750 rpm, the response of phorate was the highest too. The agitation helps sample homogenize to form liquid surface continuously. It speeds the mass transfer from liquid phase to headspace then to fiber coating. In this paper, 750 rpm of agitation speed was chosen based on described experiment.

Salt effect

Analytes that are more soluble in water have a lower affinity for the fiber coating (8). The amount of these analytes extracted by the fiber can be increased by adding sodium chloride to change the ionic strength. In order to study the effect of ionic strength on extraction efficiency, sample solutions containing 0, 10, 20, and 30% (w/v) of sodium chloride were analyzed. The extraction efficiency for most OPs was improved with the addition of sodium chloride. Only the response of bromophos methyl and phorate reached the highest point at 20% of sodium chloride, and then decreased a little when the concentration of sodium chloride was 30%. Resoponse of other OPs at 30% of sodium chloride increase a little compared with that at 20% of sodium chloride. So 30% of sodium chloride was selected in subsequent analysis.

Influence of organic solvents

Influence of organic solvents was evaluated by analyzing samples of standard solution with 5, 10, and 15% of acetonitrile. As shown in Figure 4, the amount of each OPs extracted by fiber decreases when the content of acetonitrile increases. Extraction efficiency keeps almost the same level when content of acetonitrile increases from 0% to 5%. However, extraction efficiency decreases significantly when content of acetonitrile increases afterwards. Although less content of acetonitrile improves extraction efficiency, the amount of analytes in headspace vial decreases due to dilution of analytes contained in the extraction accordingly. Therefore, 5% of acetonitrile was selected. This means that 0.75 mL of extract of acetonitrile extracted from real sample was added in headspace vial.

Desorption temperature and desorption time

Under the same extraction condition and 1 min of splitless injection, responses of analytes peaks for PDMS fiber were compared at 240°C, 250°C, and 260°C of desorption temperature. It was found that the response did not increase above 250°C, so

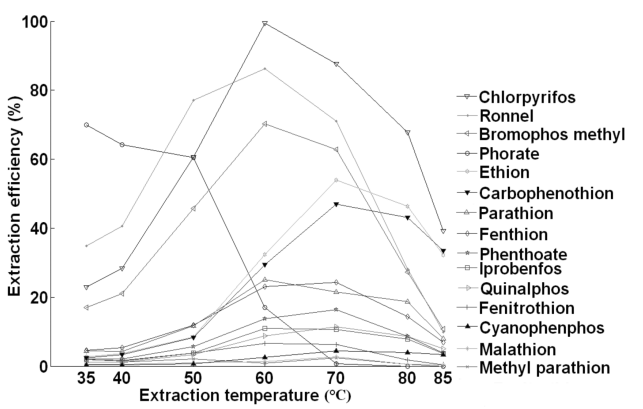

Figure 3. Effect of temperature on the extraction efficiency.The final content of individual OPs in vial is 30 ng. 750 µL of acetonitrile and 3.0 g of NaCl were added in vial and diluted to 15 mL with deionized water. Agitation speed is 500 rpm.

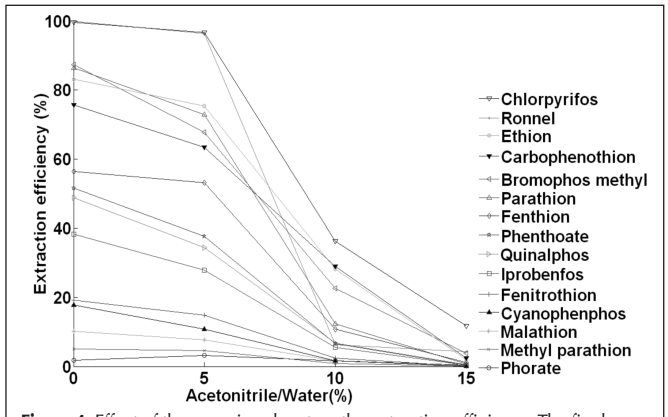

Figure 4. Effect of the organic solvent on the extraction efficiency. The final content of individual OPs in vial is 30 ng, and 4.5 g of NaCl were added in vial and diluted to 15 mL with deionized water. Extraction temperature is 70°C, extraction time is 80 min, and agitation speed is 750 rpm.

250°C was selected for desorption temperature. At this temperature, response of analytes did not change regardless of increasing splitless injection time, and therefore, splitless injection time is chosen to be 1 min. The desorption time was 6 min because no chromatogram response of residues was observed after a fiber blank run.

Injection depth was set as default of auto SPME, and there is no difference for different injection depths. In principle, a narrower inner diameter of injector liner can prevent broadening early-eluting peaks. There is no noteworthy difference between 0.75 and 4 mm i.d in the experiment. Even then, the 0.75 mm i.d. liner is still adopted in this work.

Validation of method in terms of LOD and linearity range

In order to eliminate the matrix effect, calibration curves were constructed by spiking appropriate amounts of OPs in each homogenized blank matrix of fruit samples. Figure 5 shows the chromatogram of spiked apples.

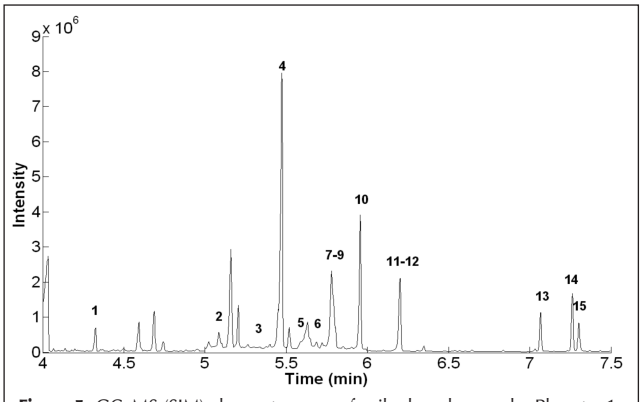

Figure 5. GC–MS (SIM) chromatograms of spiked apple sample. Phorate, 1; iprobenfos, 2; methyl parathion, 3; ronnel, 4; fenitrothion, 5; malathion, 6; fenthion, 7; chlorpyrifos, 8; parathion, 9; bromophos methyl, 10; quinalphos, 11; phenthoate, 12; ethion, 13; carbophenothion, 14; cyanophenphos, 15.

Linearity of two fruits, including apple and tomato, were evaluated according to the optimized extraction and chromatographic condition. 400 ng/g of OPs (concentration of chlorpyrifos is 200 ng/g) spiked in blank extract of each fruit was diluted with corresponding blank extract.

Due to matrix effect, linearity range of each OPs is different, and the linearity range with fast GC is lower than with conventional GC because thinner film thickness and lower column volume of microbore capillary column were adopted. Some OPs, such as chlorpyrifos, fenchlorphos, and bromophos methyl, with better extract efficiency, are easily overloaded in a microbore capillary column at higher concentrations. This results in a big tail peak or double head in the chromatogram and poor quantification. The LOD and limit of quantitation (LOQ) were calculated as the signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. The recovery was evaluated by analyzing spiked samples of 60 and 15 ng/g (concentration of chlorpyrifos is 30 and 7.5 ng/g, respectively). The results of method validation were summarized in Tables II and III. It can be seen that the precision and recovery of the method were generally satisfied.

Determination of OPs in real fruit samples by the proposed method

Apples were flushed with flowing water for 1 min. Each sample was cut into two parts of husked or not. These samples were analyzed with the proposed method, and the results are shown in Table IV. Residues of OPs were found in most samples except one apple sample. Amount of OPs decreased or could not be detected in husked samples. Tomato samples were cleaned with flowing water. Some were detected directly, whereas the others were analyzed after 60 min of rinse. Residues of OPs were found in all unrinsed tomatoes. As a comparison, the content of OPs decreased significantly or could not be detected for some samples after 60 min rinsing. It was concluded from the described experiments that residues of OPs mainly exist in pericarp of apple and tomato.

Table IV shows that residues of OPs generally exist on the surface of fruits. It is understood that the risk of OPs intake can be reduced if fruit is husked or immersed in water for some time before eating. OPs found in samples were chlorpyrifos, ethion, bromophos methyl, fenchlorphos, carbophenothion, cyanophenphos, and parathion in which contents were even lower than LOD regulated in national standard of China. LOD of OPs with proposed method was between 0.002 and 0.683 ng/g; nevertheless, the LOD of OPs with the Chinese standard method was between 0.0063 and 0.0250 mg/kg. One can see that the sensitivity is improved several hundred times with the method described here due to the higher concentration to analytes with SPME fiber and higher S/N with FGC. For example, LOD of fenchlorphos with this method is 0.002 ng/g, and the concentration of real fruit samples determined with this method is between 0.29 and 1.28 ng/g, which are lower than 0.0125 mg/kg of LOD with Chinese standard method. It means that residues of OPs detected with this

Table II. Method Validation Data in Spiked Apple Samples with Two Different Additions

Compound	Linear range	Slope	r*	LOD† (ng/g)	Added‡ (ng/g)	RSD§ (%, *n* = 5)	Recovery (%)
Phorate	12.5–400.0	152	0.9979	0.537	15, 60	5.1, 16.4	91, 86
Iprobenfos	0.78–400.0	7396	0.9979	0.062	15, 60	8.1, 8.4	108, 112
Methyl parathion	12.5–400.0	470	0.9984	0.955	15, 60	2.5, 3.4	113, 101
Chlorpyrifos	0.39–100.0	52571	0.9995	0.002	15, 60	5.7, 2.4	102, 101
Fenitrothion	12.50–400.0	1787	0.9998	0.258	15, 60	3.6, 3.3	100, 91
Malathion	6.25–400.0	2383	0.9998	0.149	15, 60	8.3, 5.5	88, 83
Fenthion	1.56–400.0	27670	0.9992	0.007	15, 60	9.2, 3.2	85, 90
Ronnel	0.20–200.0	14402	0.9997	0.008	7.5, 30	7.7, 2.9	101, 99
Parathion	3.13–400.0	6651	0.9994	0.029	15, 60	5. 6, 2.1	87, 97
Bromophos	0.39–50.00	32417	0.9985	0.004	15, 60	6.0, 3.4	105, 110
Quinalphos	0.39–200.0	14395	0.9997	0.023	15, 60	6.0, 2.5	86, 93
Phenthoate	1.56–100.0	9837	0.9988	0.013	15, 60	6.9, 2.5	83, 89
Ethion	0.39–400.0	16474	0.9984	0.009	15, 60	1.8, 7.5	102, 133
Carbophenothion	0.39–400.0	21979	0.9982	0.010	15, 60	3.8, 8.0	91, 114
Cyanophenphos	1.56–400.0	9751	0.9997	0.022	15, 60	11.9, 2.6	90, 95

* Correlation coefficient.
† Limit of detection. The LOD is 3 times S/N; limit of quantitation (LOQ) is 10 times S/N.
‡ Two different additions.
§ Relative Standard Deviation.

Table III. Method Validation Data in Spiked Tomato Samples

Compound	Linear range	Slope	r*	LOD† (ng/g)	Added‡ (ng/g)	RSD§ (%, *n* = 5)	Recovery (%)
Phorate	12.5–400.0	274	0.9990	0.586	15, 60	10.1, 20.9	101, 90
Iprobenfos	0.78–400.0	11498	0.9977	0.039	15, 60	7.1, 4.7	114, 103
Methyl parathion	12.5–400.0	814	0.9977	0.456	15, 60	6.8, 3.3	105, 100
Ronnel	0.20–100.0	56250	0.9993	0.002	15, 60	7.9, 8.0	108, 102
Fenitrothion	6.25–400.0	2624	0.9988	0.150	15, 60	10.7, 2.3	104, 97
Malathion	3.13–400.0	2982	0.9994	0.106	15, 60	11.3, 7.7	117, 102
Fenthion	1.56–200.0	26050	0.9999	0.007	15, 60	8.9, 4.0	108, 100
Chlorpyrifos	0.39–200.0	11519	0.9991	0.012	7.5, 30	7.4, 2.4	105, 100
Parathion	1.56–200.0	6311	0.9995	0.023	15, 60	7.4, 4.5	94, 110
Bromophos	0.39–200.0	32437	0.9995	0.005	15, 60	5.4, 3.5	108, 99
Quinalphos	0.78–400.0	13706	0.9994	0.025	15, 60	10.0, 4.5	113, 98
Phenthoate	1.56–200.0	9415	0.9999	0.013	15, 60	5.8, 5.4	87, 107
Ethion	0.39–400.0	10752	0.9999	0.010	15, 60	7.6, 4.5	79, 94
Carbophenothion	0.39–400.0	14803	0.9992	0.014	15, 60	5.2, 4.0	93, 93
Cyanophenphos	1.56–400.0	8691	0.9992	0.024	15, 60	6.7, 4.1	104, 97

* Correlation coefficient.
† Limit of detection. The LOD is 3 times S/N; limit of quantitation (LOQ) is 10 times S/N.
‡ Two different additions.
§ Relative standard deviation.

method may not be detected with other conventional technologies. Moreover, an analytical process with this method only took ~ 90 min; while it took ~ 6 h for the Chinese standard method to prepare a series of samples, including the process of sample extraction with organic solvents, clean-up with solid-phase extraction (SPE), and concentration. The final advantage of this method is automation of extraction avoiding tedious manual work.

Conclusion

The proposed method integrates the advantages of both automated SPME and fast GC–MS. It was proven that chromatographic condition of FGC could be translated easily from that of conventional GC by GC method translation software. Their chromatograms exhibit similar chromatographic behaviors. Organic solvents were adopted as a viable alternative to eliminate matrix effect

Table IV. Determination Results (ng/g) of Ultra Trace OPs in Two Kinds of Fruits*

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Apple															
1	–	–	–	1.28	–	–	–	2.42	–	3.83	–	–	9.34	–	–
1‡	–	–	–	–	–	–	–	0.95	–	0.42	–	–	3.38	–	–
2	–	–	–	–	–	–	–	1.66	–	–	–	–	2.65	–	–
2‡	–	–	–	–	–	–	–	0.85	–	–	–	–	2.71	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3‡	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	1.65	8.47	–	–	–	–	–	–
4‡	–	–	–	–	–	–	–	0.75	3.50	–	–	–	–	–	–
5	–	–	–	0.66	–	–	–	1.61	–	1.84	–	–	5.62	1.97	0.47
5‡	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	0.29	–	–	–	1.69	–	0.41	–	–	3.09	–	–
6‡	–	–	–	0.39	–	–	–	1.27	–	–	–	–	5.04	2.27	0.30
Tomato															
1	–	–	–	–	–	–	–	6.43	–	–	–	–	0.53	–	–
1§	–	–	–	–	–	–	–	1.51	–	–	–	–	0.36	–	–
2	–	–	–	0.96	–	–	–	1.63	–	3.41	–	–	8.29	3.10	–
2§	–	–	–	–	–	–	–	0.67	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	1.43	–	–	–	–	1.68	3.20	–
3§	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	1.48	–	2.20	–	–	1.83	2.76	–
4§	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	0.91	–	2.14	–	–	4.04	2.40	–
5§	–	–	–	–	–	–	–	0.65	–	–	–	–	–	–	–
6	–	–	–	1.29	–	–	–	18.29	–	3.83	–	–	4.62	2.30	4.77
6§	–	–	–	–	–	–	–	0.91	–	2.14	–	–	4.04	2.40	–

* 1: Phorate; 2: Iprobenfos; 3: Methyl parathion; 4: Ronnel; 5: Fenitrothion; 6: Malathion; 7: Fenthion; 8: Chlorpyrifos; 9: Parathion; 10: Bromophos methyl; 11: Quinalphos; 12: Phenthoate; 13: Ethion; 14: Carbophenothion; 15: Cyanophenphos.
† –: indicates not detected
‡ Husked samples
§ Samples dipped in water for 1 h.

on the extraction process. It could be potentially extended to other complicated matrices.

In this paper, samples of two kinds of fruits purchased from a local market in Nanchang city in China were analyzed to evaluate the proposed method. Ultra trace residues of OPs were found in most fruits. The contents were generally lower than LOD of conventional technology, and the residues mainly existed in the surface of fruits.

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