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Determination of organophosphorus pesticides in cucumber and potato by stir bar sorptive extraction

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Abstract

Organophosphorus pesticides (OPPs) in vegetables were determined by stir bar sorptive extraction (SBSE) and capillary gas chromatography with thermionic specific detection (TSD). Hydroxy-terminated polydimethylsioxane (PDMS) prepared by sol–gel method was used as extraction phase. The effects of extraction temperature, salting out, extraction time on extraction efficiency were studied. The detection limits of OPPs in water were ≤1.2 ng/l. This method was also applied to the analysis of OPPs in vegetable samples and matrix effect was studied. Linear ranges of OPPs in vegetable samples were 0.05–50 ng/g with detection limits ≤0.15 ng/g and the repeatability of the method was less than 20% relative standard deviation.

Keywords: Stir bar sorptive extraction; Solid-phase microextraction; Organophosphorus pesticides; Capillary gas chromatography; Thermal desorption; Thermionic specific detection

1. Introduction

Pesticides residues are widely presented in our environment, including water, soils, agriculture products and food. Compared to organochlorine pesticides (OCPs), the degradation rate of organophosphorus pesticides (OPPs) is much faster, resulting in widespread use of OPPs in vegetables farmland in China. To date, many methods have been developed to determine the concentration level of pesticides residues.

The sample preparation technique used for selective extraction and desorption of the target compounds are the most important steps before chromatographic analysis. Conventional methods use organic solvents in either liquid–liquid extraction or solid-phase extraction (SPE). The extracted solution is usually concentrated to 1–4 ml in volume by nitrogen purge, and 1 μl of which is utilized for chromatographic analysis, imposing stringent requirement for detection sensitivity of the analytical system. In this case, pesticide limits of detection (LODs) usually range from mg/l to tenth of μg/l for pesticides.

Solid-phase microextraction (SPME) [1,2] offers solvent-free extraction, and has been applied to pesticides analysis including OCPs [3–5], OPPs [6–9], and triazine [6,10,11]. Recently, stir bar sorptive extraction (SBSE) was developed and used for the determination of volatile and semivolatile organic compounds, including pesticides in various samples [12–17]. The extraction phases of SBSE are slices of special polydimethylsioxane (PDMS) tubing, which cover a glass tube with a magnetic core. The volume and surface area of the extraction phase are 50–200 and 400 times more, respectively, than those of SPME. The lowest detection limit with SBSE reaches sub-μg/l or even ng/l level.

The extraction phase on the stir bar in SBSE is critical for the performances of both extraction and thermal desorption. The sol–gel coating technology posses the potential to prepare thermally stable coatings [18–20]. Recently, we reported the preparation of the extraction phase on stir bar by sol–gel coating method and showed that the phase could with-

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stand 300 °C in either hydrogen or nitrogen gas atmosphere [21].

In this paper, we report the extraction and quantitative determination of OPPs in vegetables by SBSE–GC–TSD. The SBSE extraction phase hydroxyl-terminated PDMS was prepared by a sol–gel coating method developed in our laboratory.

2. Experimental

2.1. Equipment

A CP 3800 GC (Varian, USA) equipped with thermionic specified detector (TSD), was used to analyze pesticides desorbed from SBSE. A homemade thermal desorption system (TDS) was used throughout the study. Desorption temperature was 260 °C for 5 min. A centrifuge 800B (Anting Science Instrument, China) was used to clarify the sample extract.

A 5 m × 0.53 mm I.D. deactivated capillary column was connected to a 30 m × 0.25 mm I.D., 0.25 μm SE-54 analytical column by a press fit connector. The temperature of injector and detector were 260 and 300 °C, respectively. Nitrogen was used as the carrier gas at a linear velocity of 13.6 cm s^{-1}. Column oven temperature was as follows: 40 °C for 5 min, ramp to 110 °C at a rate of 30 °C/min, and then to 260 °C at a rate of 10 °C/min, hold for 20 min.

2.2. Materials

Hydroxy-terminated PDMS was obtained from Keguang New Material (Nantong, Jiangsu province, China). Methyltrimethoxysilane (MTMOS) was purchased from Danyang organic silane company (Jiangsu province, China). Poly(methylhydrosiloxane) (PMHS) was obtained from the Chemical Plant of Wuhan University (Wuhan, China). Trifluoroacetic acid (TFA) was purchased from Shanghai Chemical Plant (Chinese medicine group, China). Acetone was purchased from Chemical Plant of Shenyang (China), OPP standard mixture (1 mg/l in acetone) from J&K Acros Organics (Beijing, China). The water used in this experiment was Wahaha purified water (Hangzhou, China).

Cucumbers and potatoes were all purchased on the same day from a local market.

2.3. Preparation of sorptive stir bars

The bared glass bar (30 mm × 1.8 mm O.D.) used for the preparation of sorptive stir bar contains an iron bar inside the glass tube. The bars were sequentially cleaned by water and methylene chloride, followed by 1 mol/l NaOH and 0.1 mol/l HCl, after being washed by water, the bars were then dried at 100 °C for 2 h in a GC oven. The sol solution was prepared as follows: 200 mg of hydroxy-terminated PDMS were thoroughly dissolved in 300 μL methylene chloride; then 50 μL MTMOS and 50 mg PMHS were added. After this, 50 μL TFA (containing 5% water) were added, and vortexed quickly until the solution cleared up. After 30 min of stay, the bars were immersed into the solution for 20 min. The coated bars were then placed into a vacuum desiccator for 8 h for coating gelation. The sol–gel PDMS coated bars were conditioned in the stainless steel tube under N_2 purge. The tube was placed in a GC oven programmed from 40 to 120 °C at 1 °C/min, hold for 180 min, then to 240 °C at the same ramp and hold for 180 min, and finally to 340 °C at 1 °C/min and hold for 240 min. Before being used for extraction, the bars were purged under a N_2 stream at 300 °C for 2 h.

The coating thickness used in this experiment was about 20 μm determined by scanning electron microscopy.

2.4. Sample preparation

The concentration of 50, 100, 200, 500, 800 and 1000 ng/l of each pesticide were prepared by spiking the standards in purified water.

Ten grams of each vegetable were homogenized in a mortar, 15 ml of acetone were added and extracted for 30 min. Then, the blend was placed in a close vial and centrifuged for 5 min at 4000 rpm. Extraction was repeated twice, combined extracts were evaporated by rotarvapour to 5 ml. An aliquot of 2 ml of the concentrated extract was placed in a vial and diluted to 20 ml by purified water for SBSE.

For method validation, blank vegetable samples were spiked with OPP standards at 50, 5, 1, 0.5 and 0.25 ng/g.

3. Results and discussion

3.1. Optimization of extraction conditions

The extraction parameters, including stirring speed, extraction temperature and time, addition of NaCl, and desorption temperature and time, were optimized by using 200 ng/l solution of 12 OPPs in water.

3.1.1. Extraction temperature and stirring speed

At elevated temperature the extraction equilibrium can be reached faster but the analyte octane/water distribution coefficients (thus the extraction efficiencies) become lower. We found that the lifetime of extraction phase reduced remarkably at temperature above 40 °C. A compromise was made between lifetime of the phase and the rate of equilibrium. The following extractions were all performed at 30 °C.

Increasing the stirring rate of the sorptive bar do improve the efficiency of extraction, but may cause physical damage of the extraction phase at high stirring speed since it is in direct contact with the bottom of sample vial. We found that stirring rate at 600 rpm was adequate since further increase of the rate did not improved extraction efficiency to great extent, and will reduce the lifetime of the bars.

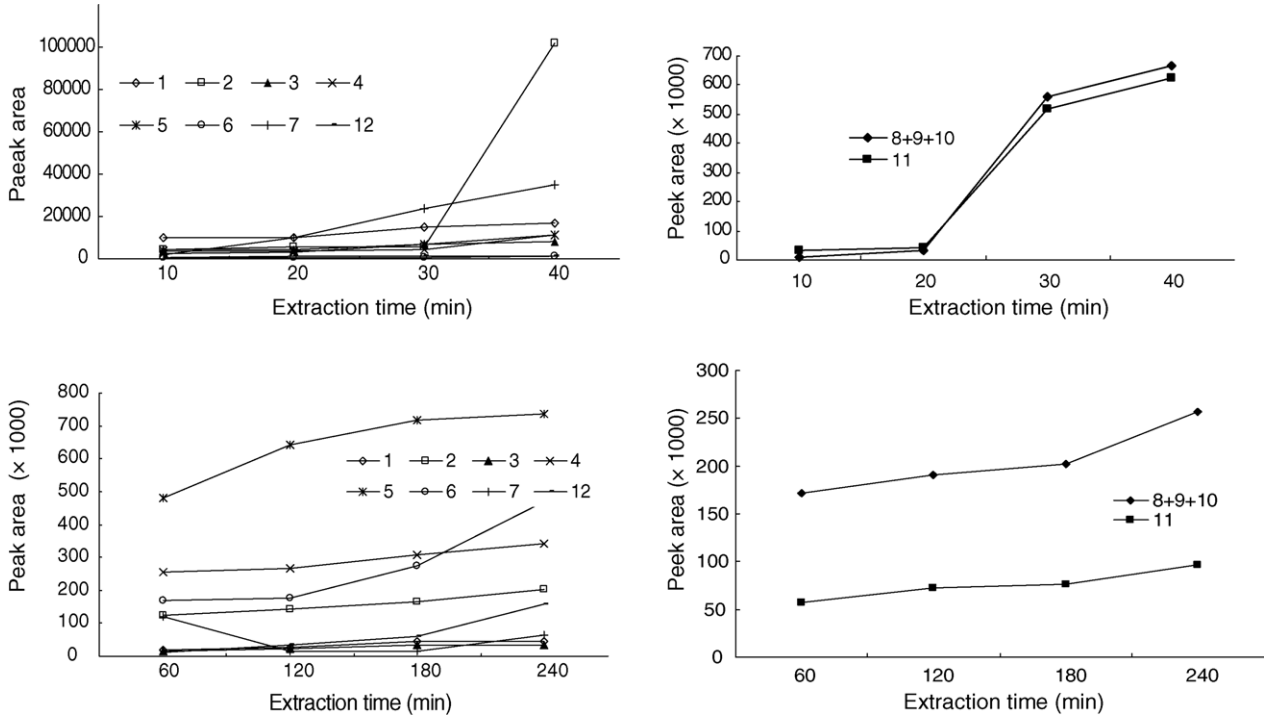

Fig. 1. Effect of extraction time on the extraction efficiency (200 ng/l OPPs). Extraction at 30 °C and stirring rate of 600 rpm; desorption at 260 °C for 5 min. 1: Monocrotophos; 2: phorate; 3: dimethoate; 4: parathion-methyl; 5: malathion; 6: fenitrothion; 7: fenthion; 8: chlorpyrifos; 9: parathion; 10: methidathion; 11: triazophos; 12: ethion.

3.1.2. Extraction time

The influence of extraction time was evaluated using a standard water solution stirred at 600 rpm at 30 °C. It was found that equilibrium of some OPPs could not be reached after 5 h of extraction, which was shown in Fig. 1. As shown in Fig. 1, the equilibrium of some OPPs could not be reached even after 5 h of extraction. Therefore, it is impractical to use the full capacity of the extraction phase in many applications. Good precision and reproducibility of extraction were obtained when the extraction conditions were strictly controlled. In this experiment 30 min of immersion was selected.

3.1.3. Salting out effect

It has been a common practice in SPME to add salt to the sample solution to enhance the extraction efficiency. We also examined the salting out effect on SBSE by adding different amounts of NaCl into the solution containing 200 ng/l OPPs. Fig. 2 shows the effect of salt concentration on the peak area of OPPs in 30 min of extraction. The extraction efficiency of most analytes increases with the increasing of salt concentration except that of monocrotophos, phorate, parathion-methyl and malathion. The highest extraction efficiency was reached at salt concentration of 30%, which was selected for later experiments.

Because the OPPs are all non-ionizable compounds in aqueous solution, the pH of the solution has little effect on the extraction efficiency. The extraction was carried out in neutral solution.

3.1.4. Thermal desorption

Thermal desorption of the OPPs from the sorptive bar was evaluated at temperature range of 240–280 °C for 5–10 min. We found that desorption at 260 °C for 5 min was sufficient and no carryover was observed.

3.1.5. Matrix effect

Previous report [22] showed that high recoveries could be achieved by using clarified sample in the determination of pesticides in apple juice, and dilution of samples was recommended to decrease the effect of matrix [23]. For vegetables, fruits and baby food samples, organic solvent such as methanol, acetone or acetonitrile are used to facilitate pesticide extraction from samples, then the extract was diluted with water prior to SBSE step [24,25].

In this experiment, acetone was used to extraction OPPs in vegetable samples before SBSE, the acetone extraction efficiency was evaluated by comparing the peak areas obtained from vegetable samples spiked prior to the acetone extraction versus matrix-matched vegetable extracts spiked at the corresponding levels (1 and 5 ng/g). The spiked samples were prepared as described in Section 2.4. The extraction efficiency shown in Table 1 ranged from 93 to 105%, from which we can know organic solvent used

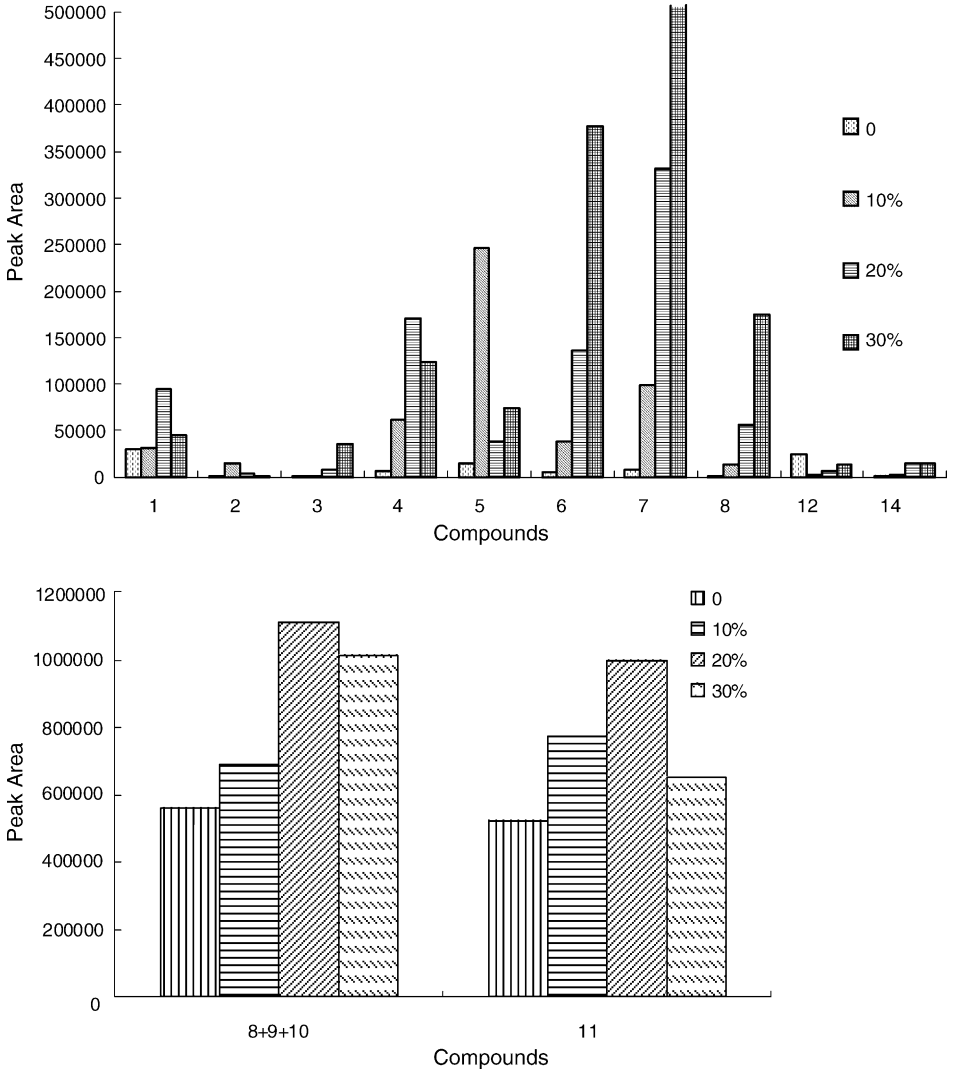

Fig. 2. Effect of NaCl concentration (0–30%, w/v) on the extraction efficiency (200 ng/l OPPs). Extraction at 30 °C and stirring rate of 600 rpm for 30 min; desorption at 260 °C for 5 min. See Fig. 1 for the list of analytes.

Table 1
Extraction efficiency of OPPs in vegetable samples

Compound	Cucumber		Potato	
	1 ng/g (%)	5 ng/g (%)	1 ng/g (%)	5 ng/g (%)
Momocrotophos	96	97	95	95
Phorate	99	95	95	98
Dimethoate	102	100	101	97
Parathion-methyl	103	102	105	101
Malathion	94	96	103	99
Fenitrothion	94	97	95	100
Fenthion*				
Chlorpyrifos*	93	97	95	95
Parathion*				
Methidathion	98	95	93	93
Triazophos	96	98	94	96
Ethion	100	99	93	95

The peaks of compounds marked * were overlapped in GC chromatogram.

in vegetable samples before SBSE is efficiency for OPP extraction.

3.2. Method validation

Based on the method development described above, the following conditions were chosen as the analytical method: the immersion time was 30 min, the extraction volume was 20 ml with stirring at 600 rpm at 30 °C, the salt concentration is 30%. For vegetable samples, acetone was used as the extraction solvent prior to SBSE.

Analyses of standard solutions with concentrations of 50, 100, 200, 500, 800 and 1000 ng/l of each pesticide were carried out to evaluate the performance of SBSE of OPPs in water. The precision was evaluated by five replicated measurements of 200 and 400 ng/l. The LODs were calculated via three times the background noise level. Table 2 shows the validation data of SBSE for water solution. A typi-

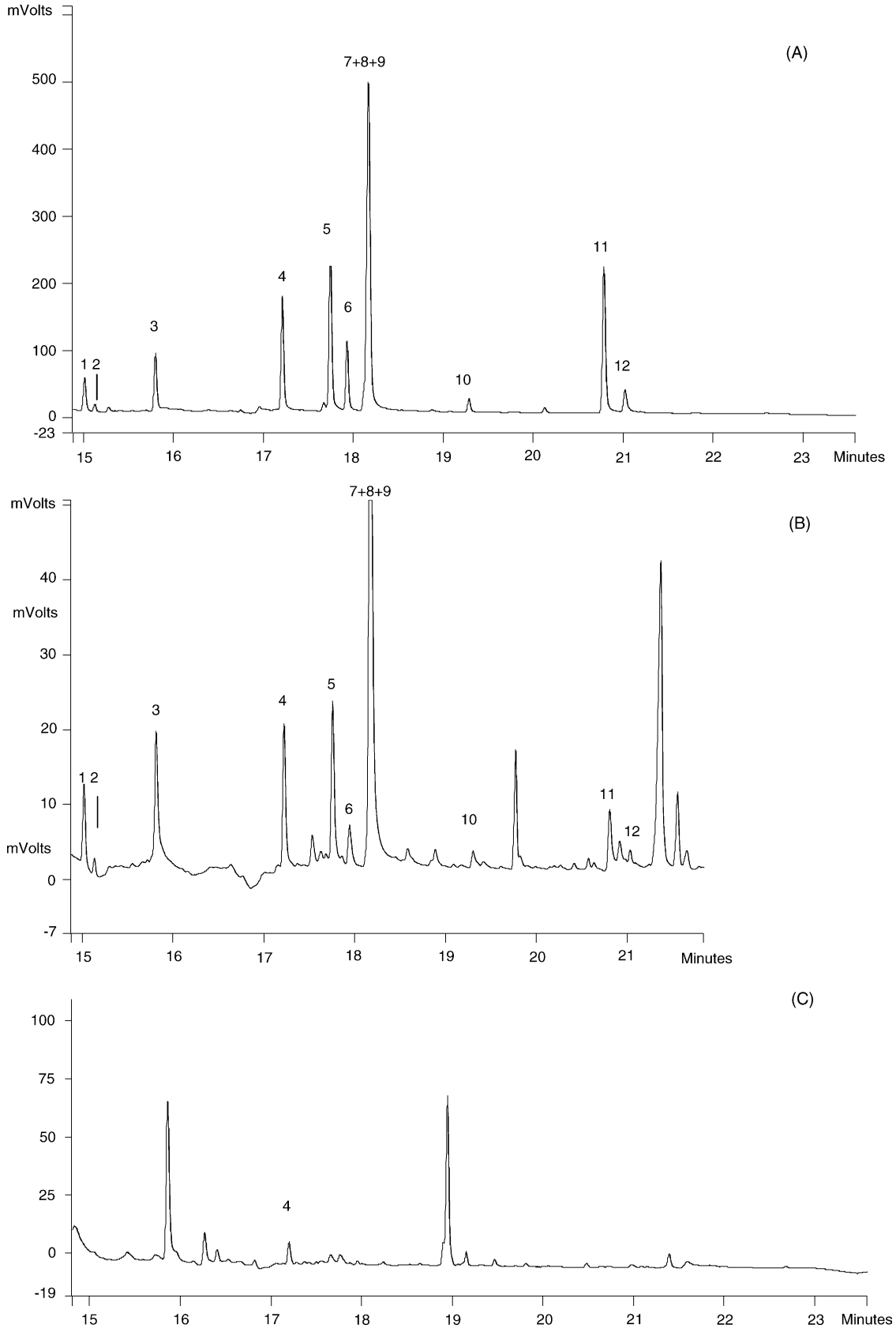

Fig. 3. GC–TSD chromatograms of OPPs obtained by the optimized SBSE method from: (A) water solution (800 ng/l); (B) spiked cucumber sample (0.5 ng/g) and (C) a potato incurred sample. See Fig. 1 for the list of analytes.

Table 2
Method validation data in standard water solutions

Compound	Linear range (ng/l)	Correlation coefficient (r)	LOD $S/N = 3$ (ng/l)	Precision RSD (%, $n = 5$)	
				200 (ng/l)	400 (ng/l)
Momocrotophos	50–1000	0.998	0.62	4	3
Phorate	50–1000	0.999	0.18	4	5
Dimethoate	50–1000	0.990	0.97	6	6
Parathion-methyl	50–1000	0.999	0.70	5	3
Malathion	50–1000	0.991	0.34	11	8
Fenitrothion	50–1000	0.990	0.42	12	6
Fenthion*					
Chlorpyrifos*	50–1000	0.991	0.06	8	3
Parathion*					
Methidathion	50–1000	0.993	1.22	6	4
Triazophos	50–1000	0.999	0.24	5	4
Ethion	50–1000	0.999	0.58	11	9

The peaks of compounds marked * were overlapped in GC chromatogram.

Table 3
Method validation data in the analysis of blank samples

Compound	Cucumber			Potato		
	Correlation coefficient (R)	LOD $S/N = 3$ (ng/g)	RSD (%, $n = 5$) 1 ng/g	Correlation coefficient (R)	LOD $S/N = 3$ (ng/g)	RSD (%, $n = 5$) 1 ng/g
Monocrotophos	0.993	0.082	6	0.998	0.052	9
Phorate	0.999	0.15	5	0.997	0.098	9
Dimethoate	0.998	0.0085	12	0.993	0.0012	6
Parathion-methyl	0.999	0.02	13	0.999	0.015	11
Malathion	0.989	0.012	28	0.995	0.013	14
Fenitrothion	0.993	0.011	31	0.999	0.008	19
Fenthion*						
Chlorpyrifos*	0.995	0.007	16	0.997	0.0056	18
Parathion*						
Methidathion	0.985	0.079	17	0.997	0.003	12
Triazophos	0.997	0.004	22	0.998	0.058	15
Ethion	0.998	0.127	14	0.998	0.091	9

The peaks of compounds marked * were overlapped in GC chromatogram.

cal chromatogram of OPPs in standard solution (800 ng/l) obtained by SBSE–GC–TSD is shown in Fig. 3A and B is a chromatogram of OPPs spiked cucumber sample (0.5 ng/g).

The blank vegetable samples spiked with OPPs standard at different concentrations were used for calibration, the linearity over a range from 0.25 to 50 ng/g was found to be good. The precision of the method was evaluated by five replicated measurements of cucumber spiked OPP standards at concentration of 1 ng/g. Table 3 shows the validation data including correlation coefficients, precision and the LODs of the analytes.

3.3. Real sample analysis

We selected cucumber and potato as representative vegetables to determine the OPPs contained in them by SBSE–GC–TSD method and Fig. 3B shows the chromatogram of a potato extract.

Dimethoate and parathion-methyl were detected in cucumbers, and in potatoes parathion-methyl was also detected, the concentration of pesticides was shown in Table 4. The levels of pesticides in two kinds of vegetables were within the maximal residue limits (MRL) regulated by EU.

Table 4
The concentration of OPPs determined in vegetables

Compounds	MRLs regulated by EU (ng/g)	Cucumber (ng/g) RSD (%) $n = 3$	Potato (ng/g) RSD (%) $n = 3$		
Dimethoate	20	9	4.0	–	
Parathion-methyl	200	5	11	2.7	10

"–" means not detected.

4. Conclusion

This study demonstrates that sorptive stir bar prepared by sol–gel technology shows good extraction–desorption properties for OPPs in vegetable extracts. The extraction phase enriches OPPs in vegetable extracts, and desorbs them completely at 260 °C for 5 min. LOD in ppt level of detection for OPPs was realized by combination of SBSE and GC. The method presented here is potentially applicable for the analysis of other GC-amenable trace contaminates in similar sample types.

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