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A quick analytical method using direct solid sample introduction and GC-ECD for pesticide residues analysis in crops

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ABSTRACT

In this work, an analytical method for GC using direct solid sample introduction was developed to tackle the problem regarding quick detection of pesticide residue in crops and large-scale screening of samples. 10 mg of the crop solid sample without sample pre-treatment was directly introduced into a modified split/splitless injector for GC analysis. A split/splitless injector was modified to quickly remove oxygen and low boiling-point matrices of the sample. The whole sampling procedure was simple and it required less than 5 min. The experimental parameters including injector-port temperature, removal of oxygen and low boiling point matrices, size and the amount of the solid sample, oven temperature program were studied. Satisfactory recoveries of 6 pesticides (methyl parathion, fenitrothion, aldrin, dieldrin, endosulfan, o,p′-DDT) were obtained in maize and rice sample. Relative standard deviation was less than 15%. Experimental results showed that the proposed method was quick and reliable for pesticide residues analysis in crops.

1. Introduction

Pesticides are being used to increase production and quality in crops over the past few decades. Excessive usage of pesticides contaminates the crop itself and the environment, eventually causing disease to human beings. China is one of the biggest crop product exporters in the world. Over 52% of the crop products are exported to countries like Japan, US and Europe. Food safety becomes a hot issue because it is not only concerning about consumers but also an international trade mark. Therefore the monitor for pesticide residue in crops is a must to the Chinese government.

A lot of analytical methods are reported in order to effectively detect the pesticide residues in crops [1,2]. Traditional pesticide residues analysis requires a large amount of organic solvent for sample extraction and a series of steps for clean-up and pre-concentration, which is complicated, tedious and expensive. The most commonly used sample preparation methods at present include accelerated solvent extraction (ASE) [3], solid-phase extraction (SPE) [4], supercritical fluid extraction (SFE) [5], solid-phase micro-extraction (SPME) [6], liquid-phase micro-extraction (LPME) [7], microwave-assisted extraction (MAE), etc. In addition, QuEChERS (Quick Easy Cheap Effective Rugged Safe) is another very popular analytical method in pesticide residue analysis [8,9]. These kinds of solvent-extraction methods have shortened the whole analytical time and enhanced the extraction efficiency. However, these methods are not simple and quick enough with regard to the problem that more and more crop-products needed to be detected.

In 2003, Shim et al. examined a Keele solid injector in GC analysis to determine vinclozolin and procymidone residues in lettuces and ginseng, respectively [10,11]. A small amount of solid sample was sealed in a glass tube and then directly introduced into the Keele solid injector for detection. Volatile flavor components in danggui ciltivars were also successfully determined by using a similar method [12]. Direct sample introduction (DSI), or named dirty sample injection, was also being studied for pesticide residues analysis in food [13–15]. Solid or liquid samples were directly transferred to a micro-vial and then introduced into a DSI injector for GC analysis. Detection by using Keele solid injector or DSI injector was quick and simple since there is no need for any sample preparation and clean-up procedure. However, they require a special injector installed on the GC system, which is not convenient and also costs extra expense for the analysis.

In the previous work, Zhang et al. have developed a new method for pesticide residue analysis in vegetables [16]. Sample without any pre-treatment was directly introduced into the split/splitless injector for GC–MS determination. This method was proven to be quick, convenient and accurate. It also worked well for rapid detection of pesticide residues in food and large-scale screening of samples in field detection. In this paper, a direct solid sample introduction for pesticide residues analysis in crops was developed based on Zhang's previous work. Little related research work was reported by using the similar technique for pesticide residue anal-

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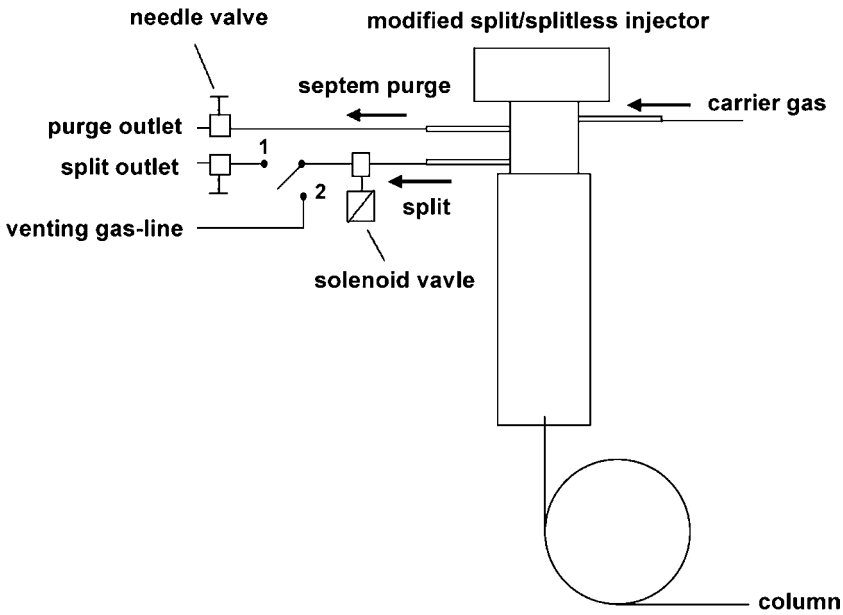

Fig. 1. Pneumatic system of the modified GC split/splitless injector. Spliting mode (1. Split outlet was connected): carrier gas goes through the split outlet with split ratio of 30:1. Venting mode (2. Venting gas-line was connected): all of the carrier gas only goes through the venting gas-line, column head pressure becomes zero.

ysis in crops. In addition, the GC split/splitless injector was simply modified to quickly remove oxygen and low boiling point matrices of the sample by adding a venting gas-line on the original pneumatic system. No sample pre-treatment was needed and the sampling procedure required less than 5 min. The injector's modification can be conducted on portable GC. Hence this method is potential for field analysis of pesticide residues in crops and large-scale screening of samples.

2. Experimental

2.1. Chemicals and materials

Pesticide standards (methyl parathion, fenitrothion, aldrin, dieldrin, endosulfan, o,p′-DDT) were purchased from Shanghai Pesticide Research Institute (Shanghai, China) with the purities from 95.4% to 99.0%. N-hexane (analytical grade) was purchased from Shanghai Ling Feng Chemical Reagent Co. Ltd. (Shanghai, China). Individual stock solution (200 μg/ml) of each pesticide standards was prepared by dissolving 2 mg of ingredient in 10 ml n-hexane. The mixed working solution of pesticide standards at different concentrations of interest was prepared by diluting the stock solution with n-hexane. All solutions were stored at 4 °C. Crop samples (maize, rice and wheat flour) were organic products from local market and were guaranteed from contamination of pesticides.

2.2. Modified GC split/splitless injector

The modified GC split/splitless injector was capable of changing the spit ratio from 30:1 to the largest within a second. An extra venting gas-line was added on the original pneumatic system of the GC (Tempcom GC7890II, Shanghai, China) as shown in Fig. 1. The system was in splitting mode when the split outlet was connected. The carrier gas would go through the spilt outlet with the split ratio of 30:1. Since the venting gas-line was connected, the system was changed into venting mode and then all of the carrier gas would go through the venting gas-line. The column head pressure became zero at the same time.

2.3. Direct solid sample introduction

5 g of the solid samples were crushed into small solid particles by using a mortar. The crushed solid samples were transferred to a 1.5 mm mesh sieve. 10 mg of the solid samples remained on the upper side of the sieve (diameter of solid sample >1.5 mm) were carefully weighted and then transferred to a glass sample vial ready for detection. The straight glass liner (78 mm long × 6.2 mm o.d. × 4.0 mm i.d.) was taken out from the injector for solid sample introduction. The pre-weighted solid sample was put in the middle of the glass liner and supported by a little bit glass wool as shown in Fig. 2(a). Finally the glass liner was installed back to the injector for GC analysis. The solid sample was disposable after each test. Next sample could be transferred into the liner for another experiment immediately. The whole sampling procedure is shown in Fig. 2(b).

2.4. GC analysis

This proposed method was performed on a GC 7890II (Tempcom, Shanghai, China) coupled with electron capture detector (ECD). A PCB-Octyl capillary column (Supelco Inc., USA) of 8 m × 0.25 mm i.d. and 0.25 μm film thickness was used. Carrier gas was N_2 (purity >99.999%) and the flow rate was 1.5 ml/min. The injector-port temperature was set at 60 °C and the injector was in splitting mode with the split ratio of 30:1 at the beginning. After the sample introduction finished, the venting gas-line was connected for 30 s to vent oxygen and low-boiling point matrices of the solid sample. The split outlet was re-connected and the injector-port temperature was immediately increased from 60 °C to 180 °C, held for 2 min, and then cooled down to 60 °C. The oven temperature program ran simultaneously from 40 °C (held for 0.5 min) to 180 °C at a rate of 8 °C/min, from 180 °C to 240 °C at a rate of 20 °C/min (held for 2 min). The detector temperature was 300 °C.

2.5. Calibration curve

A revised external calibration was used for the quantification in this method. Pesticide standards were firstly injected into the injector at an initial injector-port temperature of 60 °C. The venting

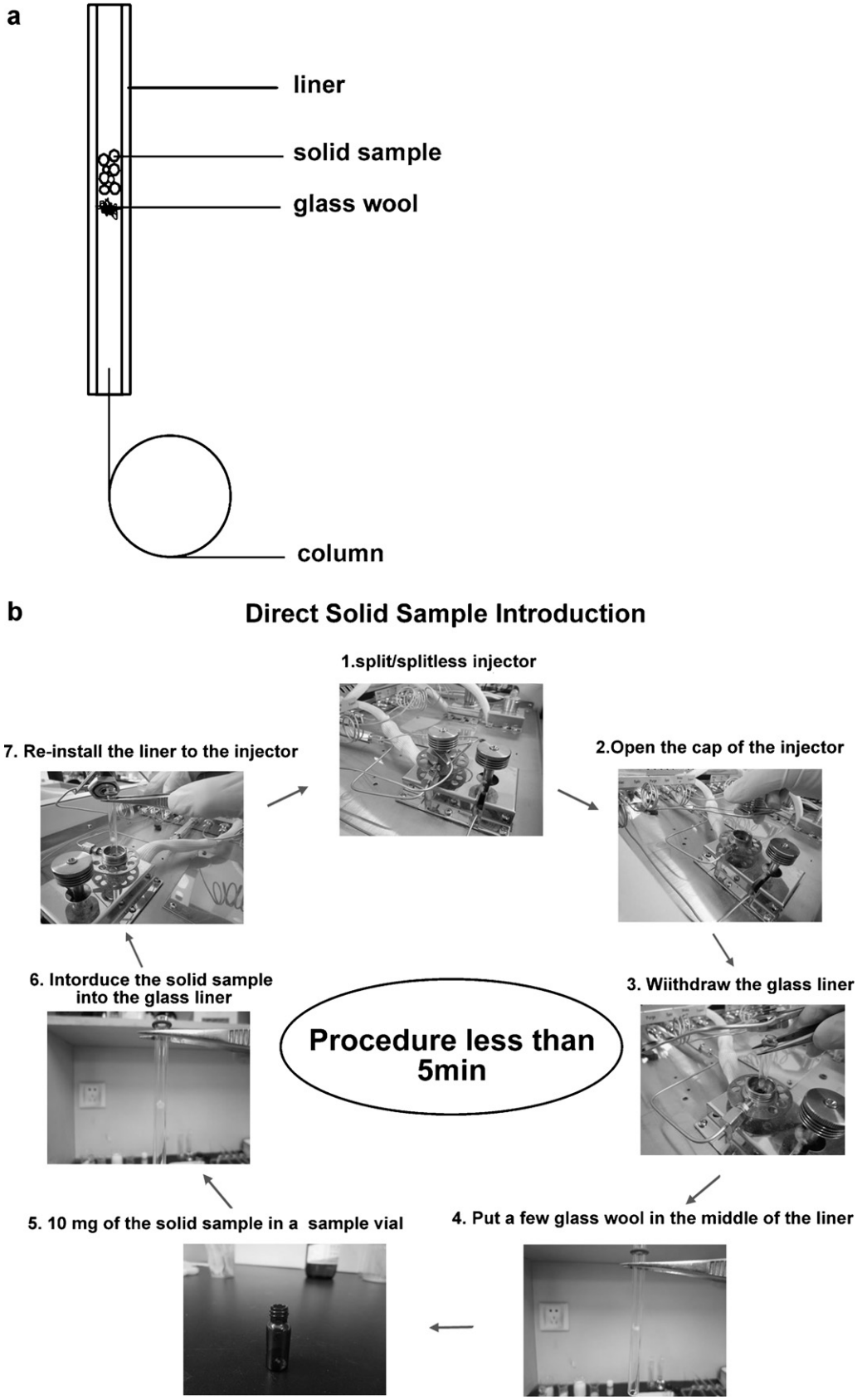

Fig. 2. (a) Schematic presentation of the solid sample introduction in the glass liner; (b) sampling procedure of the solid sample introduction.

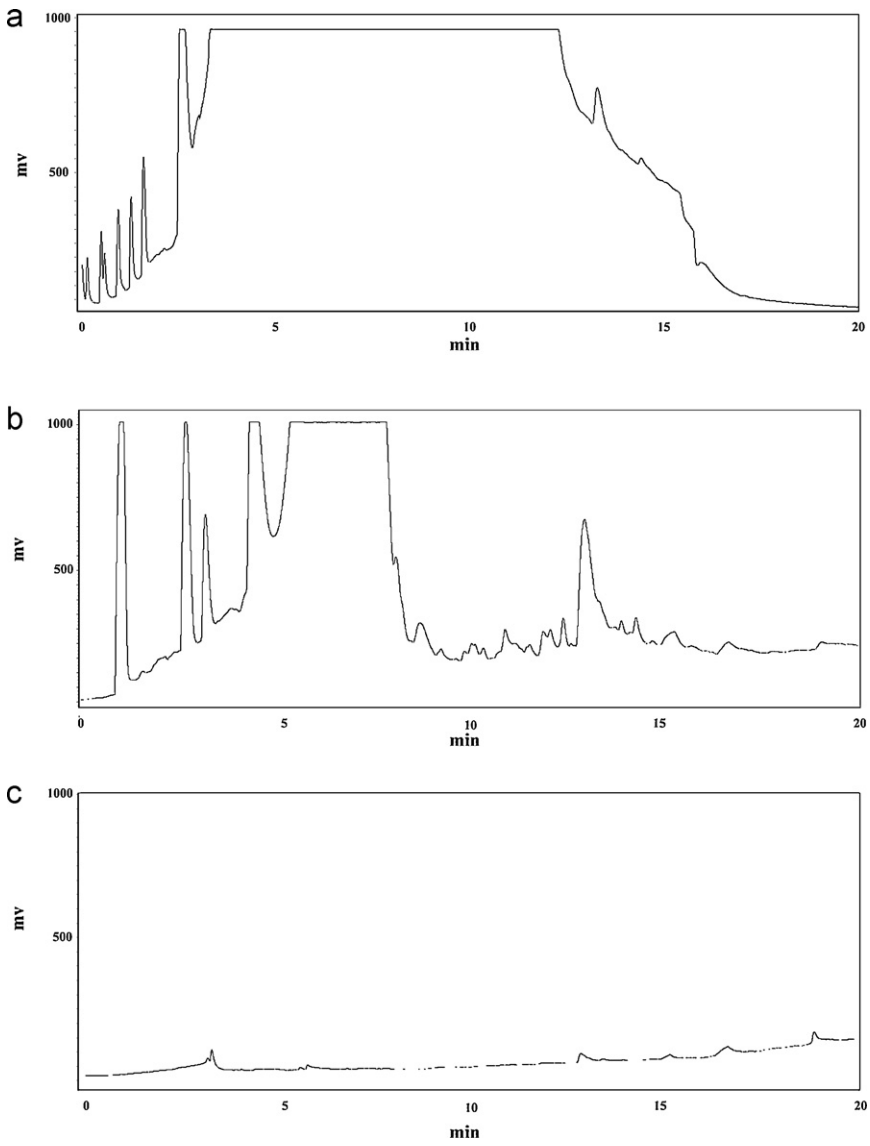

Fig. 3. Chromatograms of volatile matrices released from 10 mg of maize in different temperature condition: (a) injector-port temperature was raised to 220 °C, (b) injector-port temperature was raised to 200 °C, (c) injector-port temperature was raised to 180 °C, held for 2 min, and then cooled down to 60 °C.

gas-line was connected for 30 s, after that the spilt outlet was connected. The injector-port temperature was raised to 180 °C, held for 2 min, and then cooled down to 60 °C. The oven temperature program was started at the same time. The peak area of each pesticide was considered as the revised data. Five different concentration levels of the pesticide standards were determined to produce the calibration curve. Each concentration level was replicated for 4 times.

2.6. Recovery studies

Recovery experiments were conducted in fortified maize samples at three different concentration levels (0.01 mg/kg, 0.02 mg/kg and 0.05 mg/kg). 1 μl of the mixed working solution of pesticide standard was added into 10 mg of the sample in the liner. The sample was then put aside for 20 min for full adsorption of the solution. The liner with the sample was then installed back to the injector for recovery test. Each concentration level was replicated 5 times. The recoveries were obtained by comparing the real values of the fortified pesticide standards with calculated values.

3. Results and discussion

3.1. Optimization of the Injector-port temperature

Solid sample in the injector may release a lot of unnecessary volatile matrices at high temperature which contaminated the analytical column and detector. Besides, signals of volatile matrices severely interfered with that of the pesticides. Using maize as an example, it could be seen that the sample released a lot of volatile matrices at high injector-port temperature. Fig. 3(a) and (b) shows the signals of volatile matrices covered all signals of the targeted pesticides when the temperature was heated to 220 °C and 200 °C, respectively. It was observed that the signals of volatile matrices skyrocketed after the temperature was raised over 200 °C. It meant the boiling point of the matrices was slightly below or around 200 °C. When the final temperature was set at 170 °C or less, volatile matrices reduced but cases of incomplete vaporization of the pesticides happened on occasion. Therefore, 180 °C was selected as the injector-port temperature. Holding this temperature for 2 min guaranteed complete vaporization of the pesticides and decreasing the temperature afterward prevented the continuous vaporization

Table 1
Pesticide loss percentages under 3 different venting times (30, 60, 90 s) and 3 different initial injector-port temperatures (50, 60 and 70 °C).

Initial temp. (°C)	Venting time (s)	Pesticides loss percentages (%)					
		Methyl parathion	Fenitrothion	Aldrin	Dieldrin	Endosulfan	o,p′-DDT
50	30	8.20	4.53	9.88	3.44	10.30	8.82
	60	46.20	19.52	9.00	25.43	22.29	0.61
	90	58.51	38.65	13.39	20.89	34.24	35.97
60	30	44.90	12.94	1.38	0.58	5.83	1.02
	60	49.58	24.83	21.28	25.67	35.84	7.35
	90	62.47	49.13	8.62	17.64	30.39	28.50
70	30	47.57	30.65	4.68	9.69	26.83	24.94
	60	53.20	30.33	10.47	12.92	30.94	25.07
	90	70.34	56.67	19.80	27.87	36.93	38.76

of the matrices. Fig. 3(c) shows the chromatogram with successful reduction of the volatile matrices in maize sample.

3.2. Removal of oxygen and low boiling point matrices of the sample

During sample introduction the cap of the injector inlet have to be opened in order to withdraw or re-install the glass liner. A small amount of air might therefore enter the system. The presence of oxygen would deteriorate the analytical column and decrease the sensitivity of the electron capture detector. Therefore, removal of oxygen before analysis was needed. The modified GC split/splitless injector as described in Section 2.2 was used to overcome this problem. The GC pneumatic system was in splitting mode at the beginning. After the sample introduction finished, the system was changed into venting mode to vent the oxygen outside the injector. At the same time the column head pressure would become zero which meant nothing was entering the column. If the initial injector-port temperature was increased, some low boiling point matrices could be released from the sample and taken away by the carrier gas through the venting gas-line. However, small amount of the pesticides might be pre-vaporized in the injector and discharged via the venting gas-line, which would decrease the precision and reproducibility of the detection. Therefore, the best condition of the venting time and initial injector-port temperature was studied in order to remove oxygen and low boiling point matrices as effective as possible and retain the pesticides. Three different venting times (30 s, 60 s and 90 s) combined with three different initial injector-port temperatures (50 °C, 60 °C and 70 °C) were taken for study. Table 1 shows the loss of the pesticides under different venting time and initial injector-port temperature. Two conditions were chosen as the candidate because of the relatively minimal loss of the pesticides: (i) venting 30 s at 50 °C and (ii) venting 30 s at 60 °C. Although venting 30 s at 50 °C leaded to minimal loss of the pesticides, it was observed that more low-boiling-point matrices entered the column under this condition, which may damage the column and affect the detection. In the following experiment, decrease of sensitivity was not observed after more than 50 analyses while using 30 s as the venting time, which meant 30 s was enough for removal of oxygen. Therefore, venting 30 s at 60 °C was chosen as the optimized condition for removal of oxygen and low boiling point matrices of the sample.

3.3. Size and the amount of the solid sample

Different sizes of the solid sample may release different amounts of volatile matrices. Three sizes of maize solid samples were chosen for the comparison (diameter of the solid sample: (a) <0.15 mm, (b) 0.15–1.5 mm and (c) >1.5 mm). Experimental results presented that more volatile matrices were released when the solid sample with diameter smaller than 1.5 mm was chosen for the analysis. It is easy to understand that smaller solid sample provides larger surface area so the volatile matrices can come out from the sample much more easily. On the contrary, a lot of volatile matrices disappeared when using solid sample with diameter larger than 1.5 mm for the analysis. It proved that bigger size of the solid sample can effectively prevent vaporization of some internal volatile matrices. In addition, the amount of the sample for sample introduction was also studied. Three different amounts of the sample (50 mg, 20 mg and 10 mg) were chosen to the experiment. We found that more volatile matrices came out from the sample when 50 mg, as well as 20 mg, of the sample were used. Therefore 10 mg of the solid sample with diameter larger than 1.5 mm were finally chosen for the sample condition in this method.

3.4. Method validation

Linearity of the calibration curve was studied at a concentration ranged from 0.005 mg/kg to 0.5 mg/kg for methyl parathion and fenitrothion, 0.001 mg/kg to 0.1 mg/kg for aldrin, dieldrin, thiodan and o,p′-DDT. To consider that some of the pesticides were lost during the 30-s-venting-time, a revised calibration curve was made as described in Section 2.5. The correlation coefficients (r^2) of the revised calibration curve were over 0.997 for all the 6 pesticides. As the sample was placed directly into the liner for detection, memory effect or carry-over might be a question to the precision of this method. Recovery tests for the pesticide were used as the criteria to this problem. Recoveries were determined for 5 replicates at three different concentration levels (0.01 mg/kg, 0.02 mg/kg and 0.05 mg/kg) in maize. Satisfactory results (72–95%) were obtained in maize at 0.05 mg/kg. Decreasing recoveries for methyl parathion and fenitrothion were observed in lower concentration level. Satisfactory precision results (RSD < 15%) for all the pesticides at different concentration levels indicated that the problem of memory effect or carry-over was not serious. The reason of the decreasing recoveries for methyl parathion and fenitrothion was probably because they were strongly absorbed by the maize matrices at lower concentration (0.01 mg/kg and 0.02 mg/kg). Matrices effect to methyl parathion and fenitrothion became more obvious than that at a higher concentration level (0.05 mg/kg). Therefore a result of decreasing recovery for these two pesticides was obtained. Recoveries were also tested in the rice and wheat flour sample. Recovery results of rice (74–116%) and wheat flour (34–105%) were obtained at the concentration level of 0.05 mg/kg. Data showed the result of rice was similar to that of maize because the size of the tested rice solid samples was big (diameter of rice solid sample >1.5 mm). Fewer volatile matrices were released in bigger solid sample so the recovery was satisfactory. However, the recovery of wheat flour was bad because the size of wheat flour was very small (diameter of wheat flour solid sample <0.015 mm). Smaller solid sample would easily release volatile matrices and the matrices effect strongly decreased the recovery. Limits of determination (LOD) and limits of quantification (LOQ) were calculated by using signal-to-noise ratio of 3 and 10, respectively. LODs ranged

Table 2
Mean recovery (%) and RSD (%) of six pesticides in maize, rice and wheat flour at different concentration levels ($n = 5$).

Pesticides	Recovery % (RSD %)				
	Maize			Rice	Wheat flour
	0.01 mg/kg	0.02 mg/kg	0.05 mg/kg	0.05 mg/kg	0.05 mg/kg
Methyl parathion	53 (8)	54 (12)	74 (4)	74 (13)	34 (9)
Fenitrothion	44 (3)	77 (9)	85 (3)	93 (8)	63 (7)
Aldrin	93 (5)	90 (4)	95 (3)	108 (4)	105 (3)
Dieldrin	83 (9)	84 (5)	92 (2)	116 (8)	104 (3)
Endosulfan	81 (3)	72 (5)	75 (2)	84 (15)	60 (8)
o,p′-DDT	77 (6)	82 (9)	89 (4)	82 (11)	64 (15)

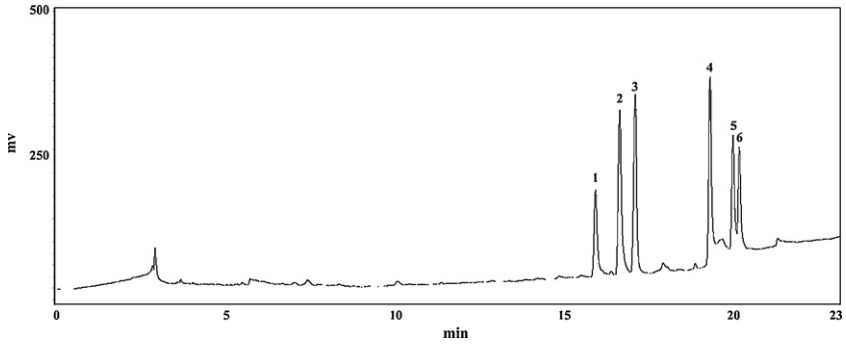

Fig. 4. Chromatogram of the 6 pesticides in maize samples (1. methyl parathion, 2. fenitrothion, 3. aldrin, 4. dieldrin, 5. endosulfan, 6. o,p′-DDT).

Table 3
LODs (mg/kg) and LOQs (mg/kg) of 6 pesticides in maize sample by calculating with S/N = 3 and S/N = 10 respectively.

Pesticides	LOD (mg/kg)	LOQ (mg/kg)
Methyl parathion	0.00072	0.00242
Fenitrothion	0.00039	0.00129
Aldrin	0.00013	0.00044
Dieldrin	0.00013	0.00041
Endosulfan	0.00020	0.00066
o,p′-DDT	0.00026	0.00086

from 0.00013 to 0.0072 mg/kg and LOQs ranged from 0.00041 to 0.00242 mg/kg were obtained in this experiment. LOD in this method is lower than the maximum residue limit for pesticide in crops established by Chinese Government. Reasonable recovery, reproducibility and precision results (RSD < 15%) in all of the samples indicated the method was suitable for the pesticide residue s analysis in crops. All of the recovery, LOD and LOQ data are shown in Tables 2 and 3. The chromatograms of the pesticides in maize samples are shown in Fig. 4.

4. Conclusions

The feasibility of a solid sample introduction method for pesticide residues analysis in crops conducted on a modified split/splitless injector was demonstrated in this paper. Experimental results showed that it was quick, simple and reliable. The modified GC split/splitless injector was able to vent oxygen and low-boiling point matrix of the sample rapidly. It was easy-equipped and could be conducted on portable GC system so the proposed method was potential in field analysis. The interference of high-boiling point matrix could be reduced by means of reasonable control of injector-port temperature. Bigger solid sample (diameter of the sample >1.5 mm) for the sample introduction would be preferable because it prevented vaporization of some volatile matrices. Therefore, the matrices effect to the pesticides was reduced. The proposed method may not surpass the existing solvent-extraction analytical method in sensitivity and reproducibility. But its quick and simple characters will make it potential in filed analysis for pesticide residue in crops. Therefore, the proposed method can be a fast screening procedure for large-scale sample so as to enhance the whole analytical efficiency.

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