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Analytical Methods

Validation of a headspace solid-phase microextraction procedure with gas chromatography-electron capture detection of pesticide residues in fruits and vegetables

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ABSTRACT

Headspace solid-phase microextraction (HS-SPME) was evaluated for the determination of pesticide residues in fruits and vegetables by gas chromatography with an electron capture detector (GC-ECD). The fibre used was coated with polydimethylsiloxane (100 μm thickness) and the analytical conditions employed have been developed and optimised in a previous work [Chai, M. K., Tan, G. H., & Asha, L. (2008). Optimisation of headspace solid-phase microextraction for the determination of pesticide residues in vegetables and fruits. *Analytical Sciences, 24* (2), 273–276]. The results show that the HS-SPME procedure gave a better linear range, accuracy, precision, detection and quantification limits and is adequate for analysing pesticide residues in fruits and vegetables. The average recoveries obtained for each pesticide ranged between 71% and 98% at three fortification levels with the relative standard deviation of less than 5%. Repeatability (0.3–3.7%) and intermediate precision (0.8–2.5%) were shown to be satisfactory. The limits of detection (0.01–1 μg L^{-1}) and the limits of quantification (0.05–5 μg L^{-1}) of these pesticides were much lower than the maximum residue levels (MRL), allowed for fruits and vegetables in Malaysia.

1. Introduction

The public awareness pertaining to the health hazards posed by pesticide residues in fruits and vegetables have led to the development of many analytical methods (Stajnbaher & Zupancic-Kralj, 2003) for measuring these residues. The main focus is on simplification, miniaturisation, and improvement of sample extraction and cleanup methods with universal microextraction procedures, namely supercritical fluid extraction (SFE) (Lehotay & Garcia, 1997; Nerin, Batlle, & Cacho, 1998; Stefani, Buzzi, & Grazzi, 1997), matrix solid-phase dispersion (MSPD) (Blasco, Font, & Pico, 2004; Bogialli, Curini, Corcia, Nazzari, & Tamburro, 2004; Kristenson, Haverkate, Slooten, & Ramos, 2001), solid-phase extraction (SPE) (Abhilash, Jamil, & Singh, 2007; Juan-Garcia, Pico, & Font, 2007; Sharif, Man, Hamid, & Chin, 2006; Stajnbaher & Zupancic-Kralj, 2003) on cartridges to replace liquid–liquid extraction (LLE), enzyme-linked immunosorbent assay (ELISA) (Bushway, Savage, & Ferguson, 1990; Watanabe, Yoshimura, Yuasa, & Nakazawa, 2001) and solid-phase microextraction (SPME) (Beltran, Peruga, Pitarch, Lopez, & Hernandez, 2003; Cai, Gong, Chen, & Wu, 2006; Chen, Su, & Jen, 2002; Zambonin, Cilenti, & Palmisano, 2002). Among these extraction and cleanup methods, SPME has become a popular technique in recent years. It is an inexpensive, environment-friendly and solvent-free technique with reliable and excellent sensitivity as well as good selectivity.

SPME was developed by Pawliszyn and co-workers in 1990 in an attempt to redress the limitations inherent in the SPE and LLE techniques (Kataoka, Lord, & Pawliszyn, 2000). It is a sample preparation technique using a fused-silica fibre which is coated on the outside with an appropriate stationary phase and is then employed to extract the analytes from a variety of matrices, which are subsequently transferred into the injector of a GC system for analysis. This sample preparation prior to the GC analysis can be carried out by direct immersion of the fibre into the sample (DI-SPME) or via the exposure of the fibre in the headspace above a liquid or solid sample (HS-SPME).

In a previous paper (Chai, Tan, & Asha, 2008), the optimisation of a HS-SPME extraction and thermal desorption procedure coupled to gas chromatography with electron capture detection for the determination of 8 pesticide residues in fruits and vegetables was carried out. A 100 μm polydimethylsiloxane (PDMS) coated fibre was found to be the most efficient in extracting the investigated pesticide residues. Parameters such as the effects of extraction time

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and temperature, salting-out effect, stirring speed, pH, desorption time and temperature, the effects of dilution and types of organic solvent were developed and optimised.

The purpose of the work reported here is to perform the validation of the optimised HS-SPME analytical method for the analysis of eight organochlorine (OC) and organophosphorous (OP) pesticide residues. The method, after validation, has been applied to various types of samples of fruits and vegetables found in Malaysia.

2. Materials and methods

2.1. Chemicals and standard solutions

All the solvents used were HPLC grade. Acetone and methanol were purchased from Fisher Scientific, Longhborough, UK. Eight pesticides standards >95% pure (diazinon, chlorothalonil, malathion, chlorpyrifos, quinalphos, profenofos, α-endosulfan and β-endosulfan) which are commonly used by local farmers in fruit and vegetable cultivation (Suzuki, 2003) were purchased from AccuStandard Inc., New Haven CT, USA. A range of standard mixture stock solutions containing 0.5–50 mg L^{-1} were prepared in methanol and stored at 4 °C. Preparation of different concentration levels of the stock solutions is carried out to correspond to the sensitivity of the ECD detector towards different compounds. Working standard solutions of a mixture of pesticides were freshly prepared daily by volume dilution in distilled water. 1-Chloro-4-fluorobenzene (98.0%), purchased from AccuStandard Inc. was used as the internal standard to compensate for sample and injection volume changes and was added to the vial prior to the GC-ECD analysis.

2.2. Sample preparation

In the multiclass and multiresidue analysis of pesticides in fruits and vegetables, three types of fruits namely strawberry (*Fragaria ananassa*), star fruit (*Averrhoa carambola*) and guava (*Psidium guajava*) and three types of vegetables namely cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*) and pakchoi (*Brassica parachinensis*) were obtained from a pesticide-free farm in the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. For the HS-SPME analysis, 100 g of the individual fruit or vegetable sample was weighed and finely chopped. A 30 g subsample was accurately weighed and placed in a 150 mL beaker. Three concentration levels – low, medium and high, were spiked into the sample to provide the spiked control sample. After being kept at room temperature for 1 h, 30 g of distilled water was added to the spiked sample which was then blended and homogenised in a food processor. The sample was then placed in separate vials.

2.3. HS-SPME analysis

A homogenised spiked sample was added with 2% (vol/weight) of methanol/acetone (1:1) and optimum dilution was made with distilled water containing 10% NaCl until the total sample in the vial was equal to 5.00 g. Then, the internal standard was added. The sample was extracted via the headspace SPME method using a 100 μm PDMS coated fibre mounted in a manual syringe holder (obtained from Supelco, Bellefonte, PA, USA) at 60 °C for 30 min; with sample agitation at 800 rpm without any pH adjustment. Desorption was done at 240 °C for 10 min.

2.4. Gas chromatography-electron capture detector (GC-ECD)

A Shimadzu GC 17A version 2.21 gas chromatograph coupled with an electron capture detector (ECD) which was purchased form Kyoto, Japan was used. A SGE BPX5, 30 m × 0.32 mm i.d. capillary column with a 0.25 μm film was used in combination with the following oven temperature program: initial temperature 120 °C, then heated at 7 °C min^{-1} to a final temperature of 250 °C, and then held for 4.5 min. The total run time was 23.07 min. A silanised narrow-bore injector liner (0.75 mm i.d.) for the SPME injections was installed and the fibre was inserted into this injector using the splitless mode. The injector temperature was held at 240 °C and the detector temperature was maintained at 300 °C. Nitrogen (99.999%) was used as the carrier gas with a gas flow at 24.4 cm s^{-1} linear velocity and the pressure maintained at 94 kPa.

2.5. Validation studies

The calibration graph of each pesticide was constructed using samples spiked with six different concentrations of the standard mixture solutions. The calibration standard mixture solutions over the concentration range of interest were prepared by serial dilution of the mixed standard stock solution with methanol and then spiked to the fruit and vegetable samples. The analyte peaks obtained were integrated and plotted as functions of the concentration. The standard mixture solutions were analysed in triplicate by GC-ECD at each concentration level.

Samples spiked at three different concentrations and three replicates for each concentration were analysed on three different occasions together with a calibration curve were performed to establish the repeatability (intra-day precision), intermediate precision (inter-day precision) and accuracy of the method. The accuracy was determined as the mean of the measured value relative to the theoretical spiked values and is reported in percentage (%). The precision is represented by the intra- and inter-day relative standard deviation (RSD).

The selectivity of the method was assessed by comparing the chromatograms obtained with and without the analytes in the blank samples. Each analyte was injected separately to ensure that no interfering peaks with the same retention times were present.

The limits of detection (LOD) and the limits of quantification (LOQ) were evaluated as the signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ in distilled water were evaluated for each pesticide as follows:

(a) Retention times were determined by running the chromatogram of a standard solution.
(b) The average noise levels were measured by running the chromatogram of a blank sample.
(c) The concentration that led to a signal of three or 10 times the noise level was evaluated using the average of the peak areas of the spiked samples in triplicate and taking into account the values of the noise level.

Recovery tests were based on the addition of known amounts of pesticides to the fruit and vegetable samples. Since the SPME technique is a non-exhaustive extraction procedure, the relative recovery, which is defined as the ratio of the concentration found in the samples and working solution, spiked with the same amount of analytes was used instead of the absolute recovery associated with an exhaustive extraction procedure. The recoveries and linearity of the method was examined on pesticide-free fruit and vegetable samples. All recoveries were determined in triplicates at three concentration levels. The different spiking levels were carried out to reflect the sensitivity of the ECD detector towards different compounds.

3. Results and discussion

The development of the HS-SPME technique for trace analysis of multiresidue pesticides in fruits and vegetables without any pre-