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Study on the Matrix Solid-Phase Dispersion and GC/MS for the Determination of Pesticides in Tobacco

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In this study, a new method for the matrix solid-phase dispersion and gas chromatography-mass spectrometry (GC/MS) determination of pesticides in tobacco was established. The tobacco samples were blended into the graphitized carbon black with a glass pestle and introduced into a polypropylene cartridge. Then, the pesticides were eluated from the homogeneous mixture with methanol as eluant. The pesticides (acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone) were determination by GC/MS with selected ion monitoring (GC/MS, SIM). The recoveries of this method were ranging from 88-96 % with relative standard deviations of 3.8-5.2 %. The detection limits were ranged from 0.05-0.10 mg kg^{-1}, respectively, for the different pesticides studied.

Key Words: Pesticides, Matrix solid-phase dispersion, Tobacco, GC/MS.

INTRODUCTION

Tobacco is one of the most important sources of cash income to the farmers and it has become one of the most commercially valued agricultural crops in the international market[1,2]. However, like other crops, tobacco also are susceptible to insect and disease attacks both in field and in storage, so pesticides are widely used for their protection[3,4]. Besides, different products, like acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone, are used to control phytophagous insects and fungal pathogens on a variety of crops[5,6]. When applied, they can also be transferred to the tobacco plantation.

In general, most methodologies for pesticide analysis in tobacco, such as the Chinese Standard Procedure, are costly, time-consuming and require larger samples and greater volumes of hazardous solvents[7]. To overcome some of the disadvantages, analytical techniques such as matrix solid-phase dispersion (MSPD) have been successfully employed. It avoids the drawbacks generally associated with liquid-liquid extraction, such as the use of large volumes of solvent, the occurrence of troublesome emulsions and slow speed[8-11]. The principle of this technique is based on the use of the same bonded-phase solid supports as in matrix solid-phase dispersion (MSPD), which is also used as grinding material for producing the disruption of sample matrix. During this procedure, the bonded-phase support acts as an abrasive and the sample disperses over the surface of the support. The MSPD technique has many applications to the processing of samples of biological origin (animal tissues, plant materials, fats *etc.*). The literature describes chromatographic methods for the determination of pesticide residues using classical sorbent material such as C_{18}-bonded silica[12-17].

During recent years, research on new materials for extraction, purification and separation processes of organic compounds in a wide polarity range has also been proposed by the growing interest for environmental preservation and human health protection[18]. Our research group have been interested in these materials because they can tailored to selective sorption profile based on hydrophobic and hydrophilic properties, shape and size of pores[19-21]. In this work, a simple, rapid gas chromatography-mass spectrometry (GC-MS) method for simultaneous determination of acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone pesticide residues in tobacco leaf. Isolation and concentration of pesticide residues from tobacco leaf was performed by matrix solid-phase dispersion with graphitized carbon black as sorbent.

EXPERIMENTAL

HPLC grade solvents, dichloromethane, ethyl acetate and chloroform, acetonitrile, methanol were purchased from Fisher Scientific Inc. (Paris, KY, USA). Certified standards of acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were at least 97 % pure. The Carbopack^{TM} B graphitized carbon black (60-80 mesh) was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Pesticide standard solutions: Stock 500 mg mL^{-1} standard solutions of pesticides were prepared by exact weight and further dissolution of the corresponding compounds in dichloro methane and stored at -18 °C. These standard solutions were stable for a period of at least 2 months. The working standard solutions were prepared by diluting the stock solutions in methanol as required. Matrix-matched standards were prepared at the same concentrations as those of calibration solutions by adding appropriate amounts of standards to the control matrix extract.

Tobacco sample preparation and fortification: A representative portion of tobacco leaf (100 g) was homogenized using a household blender and then pulverized to 200 mesh. The sample was stored in jars away from light and moisture until used for analysis. Fortified samples were prepared by adding 100 mL of a mixture of the standard solutions to 1 g of sample resulting in two final concentrations 0.2 and 0.5 mg kg^{-1} of pesticides in the sample. The fortified tobacco samples were left to stand for 0.5 h at room temperature to allow the solvent to evaporate before extraction.

A 1 g of sample was placed into an agate mortar containing 4 g of graphitized carbon black. The mixture was gently blended with a glass pestle. Once the mixture was homogeneous, it was then transferred into the top of a 2 mm × 4 mm polypropylene cartridge containing 4 g graphitized carbon black[19]. The cartridge was eluted with 20 mL of methanol and then the pesticides fraction eluent was evaporated to 1 mL by nitrogen stream. This methanol solution was filtered through a 0.45 μm syringe filter and afford for HPLC analysis.

GC/MS system and operating conditions: Agilent 6890/5973 GC-MS system (Agilent Technologies, USA) was used for the identification and quantification of the pesticides studied. A fused-silica column DB-5MS (5 % phenyl + 95 % polydimethylsiloxane; 30 m × 0.25 mm i.d., 0.25 μm), supplied by J&W Scientific (Folsom, CA, USA), was employed, with helium (99.999 % purity) as carrier gas at a flow-rate of 1.4 mL min^{-1}. The column temperature was programmed as follows: 60 °C for 1 min, then directly to 300 °C at 10 °C min^{-1} and holding for 3 min. The solvent delay was 5 min. The injector port was maintained at 250 °C and 1 μL sample volumes were injected in splitless mode (0.7 min). The data were acquired and processed with a personal computer with Agilent Chemical station software. The total analysis time was 28 min and equilibration time 2 min.

The eluent from the GC column was transferred *via* a transfer line heated at 280 °C and fed into a 70 eV electron impact ionization source, also maintained at 280 °C. The analysis was performed in the selected ion monitoring (SIM) mode. For the first acquisition window (5-10 min), the ions monitored were m/z 136, 142 and 168 (acephate). For the second acquisition window (11-20 min), the ions monitored were m/z 154, 171 and 213 (chlorpropham), m/z 152, 166 and 238 (pirimicarb). For the third acquisition window (20-28 min), the ions monitored were m/z 165, 181 and 322 (bifenthrin), m/z 227, 356 and 362 (tetradifon), m/z 121, 257 and 367 (phosalone). Values of m/z in bold type correspond to the quantification ion for each analyte.

RESULTS AND DISCUSSION

In this study, the performance of the graphitized carbon black as sorbent for matrix solid-phase dispersion was evaluated and compared with C_{18}, which was used as extracting phase to carry out the multiclass analysis of the pesticides (acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone) tobacco in our previous developed and validated MSPD procedure[22]. On the other hand, recovery experiments were carried out in 7 replicates, at 2 fortification levels (0.2 and 0.5 mg kg^{-1}) to the tobacco matrix. The recoveries from fortification studies of the 6 pesticides were evaluated by GC/MS (SIM) based on external calibration using matched standards. Average recoveries ranged from 73-89 %, with relative standard deviations (RSD) values of 4.6-6.5 % using C_{18} as sorbent and 88-96 % with RSD values of 3.8-5.2 % using the graphitized carbon black as sorbent. Concentrations were calculated by comparing peak areas from extracted ion current profiles with those obtained from matrix-matched standards. Table-1 presents recoveries of the 6 pesticides at 2 concentration levels for the tobacco leaf. Considering the acceptability criteria for recovery in the range of 70-130 %, acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone presented good to excellent recoveries from tobacco sample. Comparison of graphitized carbon black as sorbent with the commercially available C_{18}, higher recoveries and precision were obtained.

TABLE-1
AVERAGE % RECOVERIES (RSD %) OF FORTIFIED PESTICIDES IN TOBACCO SAMPLE FROM MSPD METHOD WITH GC/MS ANALYSIS

Pesticide	Fortification Level (mg kg^{-1})	Graphitized carbon black		C_{18}	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acephate	0.2	91	4.1	85	5.2
	0.5	88	3.9	89	6.2
Chlorpropham	0.2	93	4.2	84	6.5
	0.5	96	4.5	82	5.3
Pirimicarb	0.2	92	4.3	73	4.6
	0.5	91	3.8	78	4.8
Bifenthrin	0.2	89	4.6	87	5.1
	0.5	90	4.9	85	5.9
Tetradifon	0.2	88	5.2	79	4.8
	0.5	94	4.0	83	6.4
Phosalone	0.2	93	5.1	86	6.0
	0.5	95	4.3	88	5.2

The linearity of the method is a measure of range within which detector response is directly proportional to the concentration of analyte in standard solutions or samples. Linearity for all compounds were determined using blank tobacco samples fortified at 8 concentration levels (0.10, 0.20, 0.40, 0.50, 1.00, 2.00, 4.00 and 5.00 mg kg^{-1}). At each analyte amount, 2 replicate measurements were made. The slope and intercept values, together with their standard deviations, were determined using regression analyses. Linear regression coefficients for all pesticides ranged from 0.9985-0.9992. These results indicated the correct linearity of the calibration curves at the respective spiking levels. The limits of detection (LOD) for the pesticides studied were calculated considering the standard deviation of the analytical noise (a value of 7 times the standard deviation of the blank) and the slope of the regression line and ranged from 0.05-0.10 mg kg^{-1}. The repeatability of the chromatographic method was performed by successive 6 time analyses of 0.5 μg mL^{-1} of pesticide standard solution and presented as the relative standard deviations, which was in the range of 1.2-1.8 %.

Application of the method to real samples: The MSPD procedure developed was applied to the determination of pesticides in tobacco leaf. Forty six different samples of tobacco, obtained from Yunnan Province, P.R. China, originated from conventional agriculture, were analyzed using this procedure. No pesticide residues, at concentrations above the limit of detection, were found in these samples.

Conclusion

The matrix solid-phase dispersion (MSPD) was used as sample preparation method in this manuscript. Matrix solid-phase dispersion combines both sample homogenization and extraction of the analyzed compounds in one step. It considerably reduced the sample size and the solvent consumption. The method precision and recovery are higher than that of traditional solvent extraction and solid phase extraction method. Graphitized carbon black for matrix solid-phase dispersion was tested in the multiclass analysis of pesticides intobacco leaf. Results have shown that the graphitized carbon black can be successfully applied for analysis of acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone in tobacco leaf. Comparison of graphitized carbon black as sorbent with the commercially available C_{18}, higher recoveries and precision were obtained. In addition, the cost of graphitized carbon black is much lower than commercial C_{18}. In a word, this method is rapid, high sensitive and selective and provides good reproducibility and accurateness for the quantification of the acephate, chlorpropham, pirimicarb, bifenthrin, tetradifon and phosalone.

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