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Determination of Some Pesticides in Tobacco Leaf by High Performance Liquid Chromatography and Sample Preparation with Matrix Solid Phase Dispersion

YANQING YE†‡, NA WU†‡, HAITAO HUANG†‡, GUANGYU YANG†‡ and QIUFEN HU*†‡
†School of Chemistry and Biotechnology,
Yunnan Nationalities University, Kunming 650031, P.R. China
E-mail: huqiufena@yahoo.com.cn

A new method for the simultaneous determination of some pesticides in tobacco leaf by high performance liquid chromatography (HPLC) and sample preparation with matrix solid phase dispersion (MSPD) was developed. A 1.0 g of sample was placed into an agate mortar and gently blended with 4.0 g graphitized carbon black to obtain a homogeneous mixture. This mixture was introduced into a Teflon cartridge. The pesticides fraction was eluted from the cartridge with methanol and the eluant was evaporated to 0.5 mL. The pesticides (terbutylazine, phosmet, napropamide and folpet) were separated on a ZORBAX stable bound (4.6 mm × 100 mm, 1.8 μm) C_{18} column with acetonitrile and water (50:50) as the mobile phase and detected with photodiode array detector. This method provides good reproducibility and sensitivity for the quantification of terbutylazine, phosmet, napropamide and folpet. The relative standard derivations of overall intra-day variations were less than 3.8 % and the relative standard derivations of inter-day variations were less than 4.5 %. The standard recoveries (three different concentrations of markers: 0.5, 1.0 and 5.0 μg) were ranged from 88-97 %.

Key Words: RP-HPLC, Solid-phase extraction, Matrix solid phase dispersion, Pesticides, Tobacco leaf.

INTRODUCTION

Nowadays, there has been an increasing need for analytical methods for the identification and quantification of pesticides in different sample matrices such as environmental waters, vegetables, fruits, crops and others food staffs. Liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) combined with different chromatographic techniques are generally used for this purpose[1-3].

The terbutylazine, phosmet, napropamide and folpet are commonly used pesticides in tobacco planting. The determination of these pesticides by HPLC/SPME[4,5],

‡Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China.

LC/MS/DAD[6], GC/ECD(NPD)[7], GC/MS[8,9] and HPLC/DAD[10,11] had been reported. However, these methods usually need a tedious samples preparation or long time for chromatographic separation. Matrix solid-phase dispersion (MSPD) has also been successfully applied for the isolation of target molecules from biological matrices[12-16]. This procedure can considerably reduce the sample size and the solvent consumption.

The aim of this work is to set up a simple, rapid chromatographic method for simultaneous determination of terbutylazine, napropamide, folpet and phosmet pesticide residues in tobacco leaf. For the chromatographic determination, HPLC/DAD was used. Isolation and concentration of pesticide residues from tobacco leaf was performed by matrix solid-phase dispersion.

EXPERIMENTAL

The HPLC analysis was performed on a Waters 2695 Alliance separation system equipped with a 996 photodiode array detector (Waters Corporation, Milford., MA 01757, USA). The Carbopack TM B graphitized carbon black (60-80 mesh) was obtained from Sigma-Aldrich Corporation (USA).

Pesticide analytical standards were from BASF (Germany). Primary stock standard solution (1.0 mg/mL) was accurately prepared by dissolving 5.0 mg pesticide in 5.0 mL methanol for terbutylazine, napropamide, folpet and phosmet, respectively and stored frozen. Working standard mixture solution (5.0 mg/L of each pesticide) was prepared by diluting aliquot of the each stock solution with a mixture of acetonitrile:water (volume ratio 50:50) in a glass vial and stored at 4 ºC.

HPLC grade acetonitrile (mobile phase) and methanol (for sample preparation) were provided by Fisher Scientific Inc (Madison, WI 53711, USA). The ultrapure water used was obtained from a Milli-Q50 SP Water system (Millipore Inc, MA 01730 Bedford).

Chromatographic conditions: The HPLC separation were performed on a ZORBAX stable bound column (4.6 mm × 100 mm, 1.8 µm) (Agilent Technologies Inc, Santa Clara., CA 95051, USA). The column temperature is 25 ºC. The mobile phase used is acetonitrile water (50:50) at a flow-rate of 2.0 mL min^{-1}. The optimal detected wavelength was 220 nm. The sample injection volume is 20 µL. The chromatogram of pesticides standards and tobacco leaf sample at 220 nm is shown in Fig. 1.

Preparation of sample: The tobacco samples were dried by vacuum at room temperature and then pulverized to 80 mesh. A 1.0 g of sample was placed into an agate mortar containing 4.0 g of graphitized carbon black. The mixture was gently blended with a pestle. Once the mixture was homogeneous, it was then transferred into the top of a 2.0 mm × 4.0 mm Teflon cartridge (Fig. 2) containing 4.0 g graphitized carbon black. The cartridge was eluted with 20 mL methanol and then the pesticides fraction eluent was evaporated to 0.5 mL by nitrogen stream. This methanol solution was filtered through a 0.45 µm syringe filter and ready for HPLC analysis.

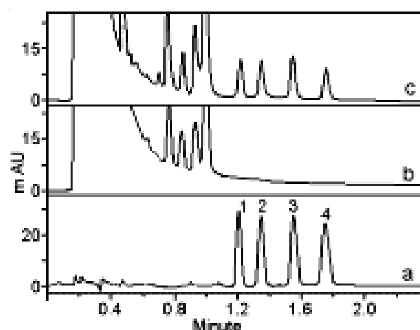

Fig. 1. Chromatogram of the standard sample (a), tobacco leaf blank (b) and tobacco samples spiked with pesticides (c). (1) terbuthylazine, (2) phosmet, (3) napropamide, (4) folpet

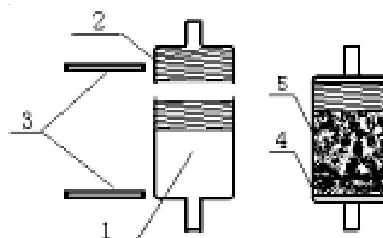

Fig. 2. The cartridge (1) Tube for fill in samples, (2) Screw cap for sealing the tube), (3) Sieve plate, (4) graphitized carbon black, (5) The mixture of sample and graphitized carbon black

RESULTS AND DISCUSSION

Matrix solid-phase dispersion (MSPD) has been successfully applied for the isolation of target molecules from biological matrices. The mechanism of MSPD includes sample homogenization, cellular disruption, exhaustive extraction, fractionation and purification into a simple process. Matrix solid-phase dispersion technology involves blending a small amount of matrix with an appropriate sorbent followed by washing and elution of compounds with a small volume of solvent. The procedure can considerably reduce the sample size and the solvent consumption. Therefore, MSPD was selected as sample preparation method in this work.

Different parameters that affect MSPD extraction such as dispersant agent and eluant solvent were studied. The polar solid phase (silica gel, alumina and florisil) and non-polar solid phase (C_{18}, graphite carbon black) were tested for matrix dispersion. High recoveries (> 95 %) were obtained when using graphite carbon black as dispersant agent. Therefore, the graphite carbon black was selected as dispersant agent and the methanol was selected as eluent in this experiment.

To evaluate the elution volume, 4, 8, 12, 16, 20, 24, 28 and 32 mL of methanol to perform elution were studied. The results show that the terbutylazine, phosmet, napropamide and folpet can be eluted from cartridge completely with 20 mL methanol used. However, further increase of the eluant volume can cause more interfering compounds eluted from the cartridge. Therefore, 20 mL of methanol was selected as eluent in this experiment.

In this study, the UV/DAD detector was used. It was set to monitor the whole range signal from 200 to 400 nm. Since the maximum absorption wavelengths were 221, 212, 222 and 220 nm for terbutylazine, napropamide, folpet and phosmet, respectively, monitoring wavelength at 220 nm was chosen to simplify data handling. Using a diode array detector, it was possible to check the purity of the peaks and to confirm the identity of the pesticides.

The optimal chromatographic condition was obtained after testing different mobile phase systems with a reversed-phase column (C_{18}). The terbutylazine, phosmet, napropamide and folpet were resolved well with a baseline separation. Furthermore, among various mobile phases examined, the mobile phase of acetonitrile and water (50:50) was found to be the best separation. Therefore, acetonitrile-water (50:50) was selected as mobile phase in this experience. To shorten the chromatographic separation time, a ZORBAX stable bound rapid analysis column (4.6 mm × 100 mm, 1.8 μm) was used in this experiment. With this rapid analysis column, the pesticides were separated completely within 4.0 min (Fig. 1). Compared to the previous literature[10,11], this is one of the most rapid methods to separation terbutylazine, phosmet, napropamide and folpet.

Under the optimum conditions, the regression equations of terbuthylazine, phosmet, napropamide and folpet were established based on the standard samples injected and their peaks area. The limits of detection are calculated by the ratio of signal to noise (S/N = 3). The results were shown in Table-1. The reproducibility of this method was also examined for 100 μg L^{-1} of the terbutylazine, phosmet, napropamide and folpet. The relative standard deviations (n = 9) were shown in Table-1.

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECT LIMIT

Components	Regression equation C (μg mL^{-1})	Linearity range (μg mL^{-1})	Coefficient	Detect limits (ng mL^{-1})	RSD % (n = 9)
Terbutylazine	$A = 6.24 \times 10^4 C - 146$	0.05-35	r = 0.9996	8	1.8
Phosmet	$A = 5.86 \times 10^4 C + 168$	0.08-40	r = 0.9992	12	2.2
Napropamide	$A = 6.87 \times 10^4 C + 208$	0.05-30	r = 0.9991	8	2.0
Folpet	$A = 8.72 \times 10^4 C + 235$	0.08-40	r = 0.9993	10	2.4

The recovery tests were carried out by adding terbutylazine, phosmet, napropamide and folpet to the samples (three different concentrations of markers: 0.5, 1.0 and 5.0 μg). The sample was prepared as above 'preparation of sample' procedure and injected for HPLC analysis to calculate the amount of the pesticides founded. The results shown that the recoveries (n = 5) were ranged from 88-97 %.

The measurements of intra-day and inter-day variability (determination of the same samples for seven times) were utilized to determine the precision of the developed method. The results show that the relative standard derivation of overall intra-day variations were less than 3.8 % and the relative standard derivation of inter-day variations were less than 4.5 %. This method is of high precision.

Conclusion

The proposed HPLC method enables simultaneous determination of terbutylazine, phosmet, napropamide and folpet because of good separation and resolution of the chromatographic peaks within 4.0 min. Compared to the routine chromatographic method[10,11], more than 70 % of separation time was saved. It is one of the

most rapid methods for chromatographic analysis of the terbutylazine, phosmet, napropamide and folpet. The matrix solid-phase dispersion (MSPD) was used as sample preparation method. MSPD 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. In a word, this method is rapid, high sensitive and selective, and provides good reproducibility and accurateness for the quantification of the terbutylazine, phosmet, napropamide and folpet.

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