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Determination of Residues of Organophosphorus Pesticides in Tobaccos by Gas Chromatography

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An optimized capillary gas chromatography (GC) method was developed to determine residues of 9 organophosphorus pesticides (OPPs) in tobaccos. The 9 OPPs were extracted by acetonitrile, cleaned-up by solid-phase extraction (SPE) column. The chromatographic separation was achieved using SE-54 capillary GC column and nitrogen phosphorus detection (NPD). The result indicated that trichlorfon, dimethoate, acephate, pyrifos, dichlorvos, methyl-parathion, methamidophos, malathion and isocarbophos were isolated well within 22 min. The standard curves for 9 OPPs showed linearity ($r > 0.99$) over the concentration range from 20 to 1000 $\mu g \cdot mL^{-1}$. The precision of 9 OPPs assay expressed as the relative standard deviation (RSD), were from 1.32 to 12.20% by five parallel determinations for the same sample. The recoveries of 9 OPPs were from 76.7 to 99.6%. The detection limit of the residues of OPPs in tobaccos was achieved at 0.01 $\mu g \cdot g^{-1}$ levels by the proposed method.

Optimisation of different chromatographic separation conditions and clean-up procedures were investigated. The validity of the method was checked by applying the standard addition technique. The method was found to be specific with good linearity, accuracy, precision and well-suited for determination of residues of OPPs in tobaccos.

Key Words: Residues, Organophosphorus pesticides, Gas chromatography, Nitrogen-phosphorus detector, Tobaccos.

INTRODUCTION

Organophosphorus pesticides (OPPs) are typically esters of pentavalent phosphorous acids, and are widely used in agriculture. To a large extent, these compounds have replaced the persistent organochlorine compounds, and are now the most frequently used group of insecticides[1]. Even if the insecticides typically act through inhibition of the enzyme acetyl cholinesterase[2, 3], they display large variation in physico-chemical properties such as polarity and water solubility.

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Now-a-days, considering accumulation of pesticides in tobaccos, the focus has been on persistent chlorinated compounds, and methods development for tobacco analysis has been targeted at this group of compounds. However, the awareness that OPPs may concentrate in tobaccos and the establishment of low maximum residue limits (MRLs) for OPPs in tobaccos as set by China and European Communities (EC), urges targeted development of methods suitable for routine screening. The objective of this work was to develop a suitable method for the determination of OPPs in tobaccos, evaluating the extract and clean-up techniques that are easy to operate.

In tobaccos, MRLs for OPPs are typically set at the level of determination (range 0.01 to 0.1 ppm)[4, 5]. To be able to analyse OPPs at this level in tobaccos, removing interfering compounds is necessary. Clean-up using gel permeation chromatography (GPC) and other techniques such as liquid-liquid partitioning, column adsorption chromatography and low temperature precipitation has been reviewed and compared[6, 7].

Nevertheless, extraction, cleaning-up, and determination of residues of OPPs in tobaccos has not been investigated. In this investigation, optimisation of extraction, clean-up and determination procedures were investigated. The validity of the method was checked by applying the standard addition technique. The method was found to be specific with good linearity, accuracy, precision and well-suited for quantitation of residues of OPPs in tobaccos.

EXPERIMENTAL

Pesiticide standards of trichlorfon (99.4%), dimethoate (99.0%), thamidophos (99.0%), malathion (97.0%), ethyl-parathion (98.8%), pyrifos (99.8%), cephate (98.8%), isocarbophos (99.4%), dichlorvos (99.0%) were purchased form Dr Ehrenstorfer (Augsburg, Germany).

Analytical-reagent grade materials were used unless otherwise indicated. Acetonitrile, petroleum ether (60–90°C), ethyl acetate, n-hexane, anhydrous sodium sulphate (pre-treated under 650°C, 2 h), and sodium chloride.

Water: glass-stilled.

OPPs stock standard solution (200 $\mu g \cdot mL^{-1}$ in n-hexane) were stored in the dark at 4°C. Working standard solutions were prepared daily.

Florisil (60–100 mesh, heated for 24 h at 130°C and brought to 3% moisture) (Floridin Co., Pittsburgh, Pa., U.S.A.), silica gel (100–200 mesh), and aluminium oxide (reagent powder).

An Autosystem XL (Perkin-Elmer, USA) gas chromatograph equipped with a split-splitless injector, a temperature programmer and a nitrogen-phosphorus detector (NPD) was used.

EB2000 Rotavapor (Beijing Analytical Instrument Co. Ltd., China), thermostated by water circulation and furnished with a vacuum pump; SP3200 ultrasonic bath (Shanghai Ultrasonic Co. Ltd., China)

Solid phase extraction columns were 15 cm × 1.5 cm i.d. Glass fitted with a coarse fritted glass disk at the bottom and a glass joint at the top to permit attachment of a 250-mL reservoir. The columns were packed (bottom upward)

with 2 g of anhydrous sodium sulphate, 15 g of florisil (or silica gel, aluminum oxide respectively), 2 g of anhydrous sodium sulphate. They were tapped gently to settle the contents and were washed with 20 mL of petroleum ether that was allowed to pass through under gravity flow until dripping nearly stopped before the sample was added.

Extraction

10.0 g ground tobacco (60–80 mesh) added with 100 mL of acetonitrile and ultrasonicate for 30 min. The macerate was filtered with suction using a Bücher funnel and Whatman No. 1 paper. After filtration through filter paper, re-extracted the filter residue with 50 mL acetone for 10 min. The extract flask was rinsed with 20 mL acetone, which was added to the residue in the funnel only after most of the initial filtrate had been collected. The filtrate was quantitatively transferred to a separator funnel with acetone rinses, 25 mL petroleum ether was added and the mixture diluted with saturated sodium chloride (10 mL) and sodium sulphate (2%, 100 mL); starting in this manner the mixture was extracted quadruple with petroleum ether which was transferred to a storage bottle. Anhydrous sodium sulphate was added to the petroleum ether extract which was stored at freezer temperature until further analysis.

Clean-up

The petroleum ether extract was then concentrated in a rotary evaporator at a low pressure at 40°C, and the extract was finally made to 2 mL and used to fill liquid-solid chromatography column. The column was eluted with ten fractions (10 mL each) of a mixture petroleum ether : ethyl acetate (20 : 80), and then discarded the first three fractions and the last two fractions, collected the other ones. Combined the collected fractions, concentrated the resulting eluate to 1 mL with a rotary evaporator. The diluting solvent contained some petroleum ether (used as standard) in order to correct instrumental variations in the quantitation process. The injected volume was 1 μL.

GC analysis

The gas-chromatographic conditions were the following: Injector temperature was 220°C. Oven temperature was isothermal at 50°C for 3 min followed by temperature programming to 250°C at 10°C·min^{-1}. The final temperature was maintained for 10 min. The split ratio was 1 : 20. The NPD was operated at a temperature of 260°C in the constant current mode with a reference current of 0.25 mV. The chromatographic column was a 30 m × 0.25 mm i.d. capillary column with SE-54 stationary phase of film thickness 0.25 μm (HP-5, Hewlett-Packard, U.S.A.). The carrier gas was hydrogen (99.999% purity) with head pressure of 80 KPa. Additional make-up gas was nitrogen (99.999% purity) with a flow rate of 60 mL·min^{-1}.

Qualitative data was verified by comparing the retention time with those of standards. Quantitative data of OPPs residues were obtained by the external method using standards as reference substances respectively, without considering calibration factors (i.e. F = 1.00 for all compounds).

RESULTS AND DISCUSSION

Extraction and clean-up

Because of the difference of the chemical structures and physical properties of OPPs, the stationary phases of gas chromatographic analysis of them were: SE-30, OV-1701, SE-54, OV-17 and so on. The OPPs investigated in this experiment involve: phosphate (dichlorvos), phosphoromonothioate (methyl-parathion, pyrifos), phosphorodithioate (malathion, dimethoate), phosphoric thioamide (methamidophos, acephate, isocarbophos)[8, 9]. In this investigation, OV101, SP-WAX stationary phase had been used, the separation effects were not satisfied. The result showed that the 9 OPPs were separated well in SE-54 column.

Florisil at 1.5, 3, 6% moisture was tested. Suitable amounts of standards were applied directly to the columns in hexane[10, 11]. Columns with added standards were eluted with 100 mL petroleum ether, followed by solvent mixtures of 20–90% ethyl acetate in petroleum ether and the fractions were analysed. Preliminary results indicated that the Forisil with 3% moisture gave the best recoveries with ethyl acetate-petroleum ether (80 : 20). Florisil (5 g, 10 g, 15 g, 20 g) was used in this investigation, and the result showed that 15 g Florisil with 100 mL petroleum ether-ethyl acetate (80 : 20) was sufficient for tobaccos analysis.

GC Analysis

Optimal conditions for the determination of OPPs was investigated. Retention times of pesticides investigated here (mean of three trials) were indicated in Table-1. Compared with traditional pesticide GC determination, it was clear that the system shows chromatograms with low drift and low noise and gives a higher sensitivity for the determination of these OPPs. This might be attributed to the better resolution of the hydrogen gas and the more effectiveness of Florisil clean-up used in this investigation.

All OPPs residues examined were quantitatively analysed under the above GC conditions by determining the sum of their peak areas. Detector responses were linear, their ranges were shown in Table-1. For each compound, the calibration curve, based on peak area measurement, was a straight line. The correlation coefficients were in the range 0.993–0.997. The limits of determination, estimated for each pyrethroid, were shown in Table-1.

Method check-up

The method was checked by using fortified samples of tobaccos. These were prepared by adding known volumes of mixed OPPs standard solution in hexane to 10-g suitable portions of ground tobaccos shown to not contain any residues of the OPPs (as shown in Table-2). The flask containing the fortified tobacco was shaken to ensure even distribution of the OPPs and, after allowing the hexane to evaporate with the aid of a gentle stream of air. The tobacco was left at room temperature for at least 2 h before extraction, in order to allow the absorption of OPPs on the tobacco and correspond more closely to a field-treated commercial

tobaccos. The samples were then extracted and determined as described in 'Procedure'. Recoveries of OPPs are 76.7–99.6% and the standard deviation of reproducibility (RSD) are 1.32–12.2%. The overall efficiency and variability was in the range reported for many residue analyses.

TABLE-1
REGRESSION EQUATION AND DETERMINATION LIMIT OF ORGANOPHOSPHOROUS PESTICIDES

Pesticide	t_R/min	Regression equation	r	Determination limit/pg
Dimethoate	4.95	y = 440x + 9125	0.999	1.3×10^{-2}
Trichlorfon	5.23	y = 2268x – 197	0.999	2.9×10^{-2}
Acephate	5.39	y = 1785x – 1032	0.999	6.4×10^{-2}
Pyrifos	5.69	y = 1481x – 1258	0.999	2.2×10^{-2}
Dichlorvos	10.58	y = 6386x – 154	0.998	1.3×10^{-2}
Methyl-parathion	12.39	y = 2934x – 8089	0.998	1.5×10^{-2}
Methamidophos	18.37	y = 468x – 48462	0.996	2.4×10^{-3}
Malathion	20.44	y = 1702x – 899	0.998	1.1×10^{-2}
Isocarbophos	21.29	y = 2364x – 1154	0.999	1.3×10^{-3}

TABLE-2
RECOVERY AND RELATIVE STANDARD DERIVATION OF METHOD

Pesticide	Adde $\mu g \cdot g^{-1}$	Recovery (%)	RSD (%)	Pesticide	Adde $\mu g \cdot g^{-1}$	Recovery (%)	RSD (%)
Dimethoate	29.80	93.97	4.56	Methyl-parathion	92.40	98.82	5.61
	2.98	83.59	3.16		9.24	80.26	12.20
Trichlorfon	13.90	95.61	1.32	Methamidophos	13.60	99.60	2.31
	1.39	76.75	2.16		1.36	80.17	4.65
Acephate	12.50	97.36	4.79	Malathion	18.50	96.04	7.26
	1.25	78.64	10.20		1.85	80.58	5.48
Pyrifos	13.90	94.90	9.12	Isocarbophos	22.00	96.81	9.13
	1.39	80.69	4.25		2.20	78.70	6.21
Dichlorvos	10.90	97.34	1.38				
	1.09	76.74	2.59				

Conclusion

Effective procedures had been developed for the extraction of residues of 9 OPPs from tobaccos, and the removal of tobacco extractives from these extracts by Florisil column chromatography to permit analysis of the OPPs at residue levels by GC-NPD. The results show that these procedures represent our current "best" solution to an analytical problem. On the basis of structural similarities, it can be assumed that this method, which had been successfully used in our laboratory for routine determinations, could be extended for the residue analysis of other OPP pesticides.

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