

Determination of Organochlorine Pesticide Residues in Tobaccos by Capillary Gas Chromatography

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A simple, rapid, relative reliable method for extraction of 17 organochlorine pesticides in tobaccos and their determination by capillary gas chromatography was presented. The pesticides were extracted and cleaned-up by a Soxhlet extractor with hexane as extracting solvent. Different methods were compared for the determination of organochlorine pesticides in tobaccos. The Soxhlet extraction was evaluated for organochlorine pesticides analyses in tobaccos while liquid-liquid partition and solid phase extraction methods were compared. Of all the extracting methods used, Soxhlet extraction gave the best results.

Key Words: Simultaneous extraction cleaning-up, Organochlorine pesticide residues, Capillary gas chromatography, Electron capture detection, Tobaccos.

INTRODUCTION

The number of pesticides that are routinely used in agriculture to both lessen the detrimental effect of weeds, insects or diseases, and boost crop production, has dramatically increased in recent years. As a consequence, the determination of pesticide residues in crops has been strictly regulated by governments in all countries, with two basic aims, namely to detect the presence of forbidden pesticides on a particular commodity and to determine whether the concentrations of pesticides used exceed their maximum residue limits (MRLs)^{1, 2}. The determination of pesticide residues in tobaccos requires simple, rapid, efficient methods because of the short time between harvest and sale in markets. Undoubtedly, organochlorine pesticides (OCPs) constitute one of the most important groups of hazardous organic contaminants owing to their high persistence and impact on human health³. Despite the ban and restriction on the usage of OCPs during the 1970s and 1980s, some developing countries are still using them for agricultural purposes because of the low cost⁴. Studies have suggested that these compounds may affect the normal function of the endocrine system⁵. Similar studies have

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indicated the presence of contamination by OCPs in soil and water⁶, and noticeable concentrations have been found in these regions. Studies of soil and water monitoring for OCPs in developed European⁷, Asian⁸ and American⁹ countries have shown widespread detection of these pesticides in ground and surface waters where they have been banned for decades. In developing countries such as China, this class of pesticide is believed to be still in use clandestinely under different trade names due to its cheapness. In certain slum areas, it has been used for malaria control. However, there is still a paucity of data on OCPs in tobaccos.

Several extraction and cleaning-up methods have been developed and applied for chromatographic separation and detection of OCPs. Common methods include liquid-liquid partition (LLP)¹⁰⁻¹² and solid-phase extraction (SPE)^{12,13}. Solid phase microextraction techniques (SPME) have also been applied¹⁴. The unique physical properties of supercritical fluids extraction (SFE) is described to provide cleaner extracts, less solvent handling, and equivalent or better recoveries than conventional solvent extraction techniques¹⁵. Supercritical CO₂ is the most commonly used fluid for SFE because of its low critical constants, its low toxicity and its ability to extract wide range of non-polar organics from varieties of matrices. However, extraction of polar and ionic analytes requires the addition of organic modifiers to CO₂¹⁶. The use of SFE techniques for the extraction of OCPs has been widely reported¹⁷. Despite the fact that SFE of OCPs has shown remarkable advantages over solvent extraction techniques, there are indications that this technique is not completely successful yet especially for biotic matrices (*i.e.*, tobacco samples).

The use of capillary GC coupled with ECD for the detection of OCPs is common because of its high resolution and good sensitivity in the nanogram range¹⁸. Other advantages of this detector include reduced cost of operation and the fact that it requires less technical skill to obtain reliable results. It has better resolution and could give higher sensitivity than the ordinary GC method.

Solvent extraction is a common method frequently used for the determination of organic pollutants¹⁹. It has frequently been considered to give more reliable data than SPE (the use of commercially available SPE cartridges for sample preparation in OCPs analyses has been shown to give rise to interferences specially when GC-ECD is employed). Tan¹⁰ in his study indicated that solvent extraction for sample enrichment of OCPs would give more repeatable data. In this study, the efficiency and repeatability of the Soxhlet extraction was evaluated for the determination of 17 organochlorine pesticides in tobaccos. The result showed that Soxhlet extraction was a simple method for determination of organochlorine pesticides in tobaccos.

EXPERIMENTAL

A Shimadzu GC-14B (Shimadzu, Japan) gas chromatograph equipped with a split-splitless injector, a temperature programmer and a ⁶³Ni electron-capture detector (ECD) was used. In all cases the chromatograms were recorded by using a Shimadzu CR-7A (Shimadzu, Japan) computing integrator. EB-2000 Rotavapor

(Beijing Analytical Instrument Co. Ltd., China), thermostated by water circulation and furnished with a vacuum pump.

Analytical-reagent grade materials were used unless otherwise indicated.

α -BHC, β -BHC, γ -BHC, δ -BHC, Heptachlor, Aldrin, Heptachlor-epoxide, α -Endosulfan, 2,4'-DDE, 4,4'-DDE, dieldrin, endrin, β -endosulfan, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, and *trans*-chlordane (Analytica, Sigma) stock standard solution (200 $\mu\text{g mL}^{-1}$ in *n*-hexane) were stored in the dark at 4°C. Working standard solutions were prepared daily.

n-Hexane for pesticide analysis, used as an extraction and cleaning-up solvent, was obtained from Fluka (Buchs, Switzerland) and Florisil (60–100 mesh) from Floridin Ltd. (Floridin Co., Pittsburgh, Pa., U.S.A.).

Florisil was heated overnight in a muffle furnace at 500–550°C, then cooled in a desiccator without a desiccant. For deactivation, florisil was placed in a round-bottomed flask on a rotary evaporator. With constant agitation, 5 wt % of doubly distilled water was added and the flask rotated for 1 h. before use; the deactivated florisil was left to equilibrate for at least 48 h in a rightly closed glass container. After each use, the glass container must be tightly reclosed in order to prevent modification of the water content deactivated florisil.

The Soxhlet extractor was filled with 5.0 g of deactivated florisil as the lower layer and 5.0 g of deactivated florisil mixed with 5.0 g of dry ground tobacco (40–60 mesh) as the upper layer²⁰.

A 60 mL volume of *n*-hexane was placed in a 250 mL round-bottomed flask. The flask was connected to the Soxhlet extractor and the *n*-hexane was heated with an electrical heater to boil the solution. The Soxhlet extractor was regulated to give a reflux rate of *ca.* 250 mL h⁻¹. The level of *n*-hexane above the tobacco had to be kept constant. The overflow prevented drying of the distillation flask. The extraction time was 6 h. After extraction, concentrated the resulting extract to 1 mL with a rotary evaporator; 1.0 μL of the extract was injected directly into the GC system.

The gas-chromatographic conditions were the following: Injector temperature was 280°C. Oven temperature was isothermal at 60°C for 2 min followed by temperature programming to 150°C by 5°C min⁻¹, then to 270°C by 2°C min⁻¹. The final temperature was maintained for 5 min. The split ratio was 1 : 50. The ECD was operated at a temperature of 300°C in the constant current mode with a reference current of 1.0 nA. The chromatographic column was a 30 m \times 0.25 mm i.d. capillary column width of 0.25 μm film thickness (BD-5, J & W Scientific, Folsom, CA, USA).

The carrier gas was hydrogen (99.999% purity) with flow rate of 80 mL min⁻¹. 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 OCPs were obtained by the external method using standards as reference substances respectively.

RESULTS AND DISCUSSION

Extraction and clean-up

The non-specific nature of the electron capture detector introduced the requirement that methodology be developed to provide interference free tobacco samples. A review of available methods for the determination of OCPs residues led to the conclusion that these matrices were among the most troublesome¹. The procedures used were tedious and time-consuming, and involved several extraction and clean-up steps to remove the huge amount of potentially interfering compounds (mainly plant pigments etc.), which were generally present at higher concentrations than the pesticide residues themselves. The large volumes of organic solvents employed in sample preparation (typically acetone, acetonitrile and ethyl acetate), 50–200 mL, posed major hazards for both the environment and the operator.

This work was aimed for developing a simple method involving minimal sample manipulation, for the screening and sensitive and selective determination of OCPs in tobaccos. Solvent extraction of analytes from ground sample is unavoidable, and is simultaneously cleaned-up. For this purpose, a continuous overflow system²⁰ was suited to the proposed application. The method was simple, rapid, reliable and highly sensitive; it allowed simultaneous extraction, cleaning-up and quantitation of up to 17 OCPs residues in amounts below their maximum residue limits. In addition, the high efficiency of the Soxhlet procedure provided good blanks and avoided the interference of coextractives.

Compared with soxhlet extraction, liquid-liquid partition extraction sometimes resulted in emulsification, which could lower the accuracy and reproducibility of the analytical results (Table-1). In this regard, SPE is an effective tool for cleaning-up purposes, but the recoveries were not satisfied in tobacco samples as shown in Table-1. Of all the extracting and cleaning-up methods used, soxhlet extraction gave the best results^{11, 12}.

Capillary GC Determination

The DB-5 poly (5%) diphenyl (95%) dimethylsiloxane was used in the GC-ECD determination of OCPs in this study because of its good resolution and its good retention values for the OCPs¹⁸. Optimal conditions for the determination of OCPs were investigated. Retention times of pesticides investigated here (mean of three trials) are indicated in Table-1. Compared with LLE flowed by SPE of OCPs GC determination²¹, it was clear that the system shows chromatograms with low drift and low noise, gives a higher sensitivity for the determination of these OCPs. This might be attributed to the better resolution of the hydrogen gas used as carrier gas²². All OCPs residues examined were quantitatively analyzed under the above GC conditions by determining the sum of their peak areas. Detector responses were linear; their ranges are shown in Table-3. For each compound, the calibration curve, based on peak area measurement, was a straight line. The correlation coefficients were in the range 0.992–0.998. The limits of determination, estimated for each OCP, were shown in Table-2.

TABLE-1
RECOVERIES (R%)^a AND THEIR RELATIVE STANDARD DERIVATION (RSD%) OF
OCPs IN TOBACCOS EXTRACTED BY SOXHLET, LLP AND SPE

Pesticide	Spiked at $\mu\text{g g}^{-1}$	Soxhlet		LLP		SPE	
		R%	RSD%	R%	RSD%	R%	RSD%
α -BHC	2.0	106.30	5.59	111.30	15.5	71.05	11.50
β -BHC	2.0	99.82	7.56	69.21	17.6	62.02	9.29
γ -BHC	2.0	96.15	5.12	76.52	25.1	72.18	6.64
δ -BHC	2.0	83.14	9.78	103.40	19.7	44.29	10.70
Heptachlor	0.2	84.31	9.56	74.25	19.5	68.15	11.50
Aldrin	0.2	94.14	5.64	104.10	25.4	74.28	9.29
Heptachlor-epoxide	0.2	99.26	5.98	109.30	15.8	69.31	8.78
<i>trans</i> -Chlordane	2.0	95.16	5.13	115.70	11.6	55.25	9.25
2,4'-DDE	10.0	75.14	8.16	69.32	18.1	49.13	10.10
α -Endosulfan	0.2	101.40	5.46	121.70	10.4	61.14	6.35
Dieldrin	0.2	84.22	9.54	74.32	12.3	54.23	10.20
4,4'-DDE	2.0	91.81	5.45	101.20	11.4	41.85	9.37
Endrin	10.0	87.11	5.46	79.33	23.4	37.11	5.46
β -Endosulfan	10.0	109.50	12.70	121.90	20.9	59.53	11.23
2,4'-DDD	0.2	88.14	7.45	68.23	27.1	48.18	9.34
4,4'-DDD	0.2	91.56	9.05	111.40	10.5	51.26	12.80
2,4'-DDT	0.2	85.23	9.86	65.54	18.6	45.19	13.50

^aValues are mean of triplicate analysis

Method check-up

The method was checked by using fortified samples of tobaccos. These were prepared by adding known volumes of mixed OCPs standard solution in hexane to 10 g suitable portions of ground tobaccos shown not to contain residues of OCPs (Table-2). The flask containing the fortified tobacco was shaken to ensure even distribution of OCPs 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 for OCPs to be absorbed on the tobacco and correspond more closely to field-treated commercial tobaccos. The samples were then extracted and determined as described in procedure. Recoveries of OCPs were 75.14–106.3% and the standard deviation of reproducibility (RSD) was 5.13–12.7%. Finally, as an example of routine determination, the overall efficiency and variability was in the range reported for many residue analyses.

TABLE-2
REGRESSION CURVE EQUATIONS AND CORRELATION COEFFICIENTS (r)
FOR THE OCPs

Peak No.	Pesticide	Retention time (t/R, min)	Regression equation	r	Detection limit (ng g ⁻¹)
1.	α -BHC	12.9	$y = 2.73 \times 10^5 x + 8.6 \times 10^3$	0.998	20.00
2.	β -BHC	13.3	$y = 1.79 \times 10^5 x + 4.6 \times 10^3$	0.992	9.68
3.	γ -BHC	14.8	$y = 2.80 \times 10^5 x + 9.9 \times 10^3$	0.993	17.10
4.	δ -BHC	16.5	$y = 2.99 \times 10^5 x + 6.8 \times 10^3$	0.996	15.20
5.	Heptachlor	19.6	$y = 2.51 \times 10^5 x + 10.3 \times 10^3$	0.989	12.40
6.	Aldrin	22.1	$y = 2.73 \times 10^5 x + 10.8 \times 10^3$	0.987	17.20
7.	Heptachlor-epoxide	25.3	$y = 2.70 \times 10^5 x + 9.4 \times 10^3$	0.991	20.30
8.	<i>trans</i> -Chlordane	27.3	$y = 2.61 \times 10^5 x + 9.5 \times 10^3$	0.998	21.20
9.	2,4'-DDE	28.2	$y = 2.51 \times 10^5 x + 9.9 \times 10^3$	0.996	7.98
10.	α -Endosulfan	28.6	$y = 2.76 \times 10^5 x + 9.1 \times 10^3$	0.991	25.30
11.	Dieldrin	30.4	$y = 2.53 \times 10^5 x + 9.5 \times 10^3$	0.997	5.17
12.	4,4'-DDE	30.8	$y = 2.98 \times 10^5 x + 8.5 \times 10^3$	0.992	6.79
13.	Endrin	32.1	$y = 2.49 \times 10^5 x + 8.6 \times 10^3$	0.994	22.50
14.	β -Endosulfan	34.7	$y = 2.26 \times 10^5 x + 4.6 \times 10^3$	0.992	11.60
15.	2,4'-DDD	36.8	$y = 1.99 \times 10^5 x + 3.5 \times 10^3$	0.994	21.30
16.	4,4'-DDD	37.6	$y = 2.44 \times 10^5 x + 7.5 \times 10^3$	0.992	17.70
17.	2,4'-DDT	40.5	$y = 1.33 \times 10^5 x + 1.7 \times 10^3$	0.995	18.90

OCPs residues in tobaccos

This method was found to be effective for simultaneous determination of 17 OCPs residues in tobaccos. The 17 OCPs examined in this study were monitored by the proposed method of multi-residue analysis in 9 tobacco samples from 3 different areas: Guizhuo, Yunnan and Henan. The limits of detection for OCPs ranged from 5.17–25.3 $\mu\text{g g}^{-1}$ by the proposed method. The suggested method was applied to tobacco samples from China. The levels of OCPs ranged from 0.004 (α -BHC) to 0.248 $\mu\text{g g}^{-1}$ (endosulfan) in tobaccos. Some endocrine disrupting OCPs such as DDT, DDE, heptachlor, endosulfan and the chlordanes were detected in the samples. The results showed that the different OCP and amount were obtained in different tobaccos. Therefore, this method could be applied for rapid OCPs residue screening in tobacco imports and exports qualities.

Conclusion

Effective procedures had been developed for the extraction of OCPs residues from tobaccos, and Soxhlet extraction of tobacco extractives to permit analysis of OCPs at residue levels by GC-ECD. These procedures represent our current

best solution to an analytical problem. On the basis of structural similarities, one could assume that this method, which had been successfully used in our laboratory for routine determinations, could be extended for the residue analysis of OCPs in other plant samples.

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