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Determination of pyrethroid residues in tobacco and cigarette smoke by capillary gas chromatography

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Abstract

The extraction of fenpropathrin, cyhalothrin, cypermethrin, fenvalerate and deltamethrin from tobacco (*Nicotina tobaccum*) and cigarette smoke condensate with acetone, followed by partition of resulting acetone mixture with petroleum ether, was investigated and found suitable for capillary gas chromatography (GC) residue analysis. Florisil column clean-up was found to provide clean-up procedure for tobacco and cigarette smoke condensate permitting analysis to $\leq 0.01\ \mu g \cdot g^{-1}$ for most of the pyrethroids by GC with a ^{63}Ni electron capture detector (GC–ECD). Quantitative determination was obtained by the method of external standards. Cigarettes made from flue-cured tobacco spiked with different amounts of pyrethroids were used and the pyrethroid levels in mainstream smoke were determined. For all the pyrethroid residues, 1.51–15.50% were transferred from tobacco into cigarette smoke. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Tobacco smoke; Pyrethroids

1. Introduction

Pyrethroid pesticides are substances of intense interest for use in tobacco protection because of their desirable environmental properties of short persistence and nontoxicity to mammals [1]. These features combined with their broad spectrum of pesticidal activity have made the pyrethroids alternatives to the older organochlorine compounds and the natural pyrethrins. The rapid increase in their application and the recent commercial introduction of some new pyrethroids require a method by which the residue of many pyrethroids can be determined. These compounds were easily analyzed by gas–liquid chromatography with electron capture detector (GLC–ECD) because of their thermal stability and electron capturing properties [2]. However, the non-specific nature of ECD required that a methodology be developed to provide interference-free extracts [3]. To this end, the present method liquid–solid chromatography (i.e. Florisil column) was used for clean-up step. Liquid–solid chromatography was an efficient sample clean-up technique based on separation mechanisms of liquid chromatography. Suitable clean-up procedures for GC analysis of pyrethroid residues in vegetables using ECD had been reported [4,5]. GLC and high-performance liquid chromatographic methods had been developed for the single- and multi-residue determinations of pyrethroids [6–18].

To prevent excessive treatment of tobaccos with

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pyrethroids, several countries have introduced maximum limits for pyrethroid residues. In view of these legal measures, industrial laboratories have made considerable efforts to analytically assess pyrethroid residues in tobacco and cigarette smoke. This study investigated the fate of pyrethroids during smoking, in particular their transfer rate from the tobacco into the smoke or the collection shifting efficiency (CSE). There are two sets of data that are essential for the determination of the suitability of pyrethroids for use on tobaccos. These are the amount of pyrethroid residues in tobacco and their transfer rate into cigarette smoke. The first is required to meet the pyrethroid tolerance imposed by different tobacco-growing and tobacco importing and exporting counties. Extensive work had been done on this aspect. The second set of data is required for assessing the hazard that the pyrethroids used may pose to the smoker. Several, though not enough, publications had appeared which give such information (see Chopra et al. [20] and references cited therein). Further information on this aspect is of great importance. Though studies on the pyrethroid residues in tobacco had been reported, nothing to our knowledge had been published on the pyrethroid residues in cigarette smoke. In this paper, we attempted to meet that need.

This study reports our observations on the extraction of fenpropathrin, cyhalothrin, cypermethrin, fenvalerate and deltamethrin from tobacco and cigarette smoke condensates, their elution from Florisil and the utility of these procedures for analysis of pyrethroid residues by GC–ECD.

2. Experimental

2.1. Apparatus

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

A Borgwaldt RM 20/CS smoking machine (HB, Germany) was used to collect mainstream cigarette smoke condensate.

A EB2000 Rotavapor (Beijing Analytical Instrument, China), thermostated by water circulation and furnished with a vacuum pump, was used for concentrating. A SP3200 ultrasonic bath (Shanghai Ultrasonic, China) was used for extraction.

Liquid–solid chromatography columns were 15× 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 sulfate, 15 g of Florisil (or silica gel and aluminium oxide), 2 g of anhydrous sodium sulfate. 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.

2.2. Reagent

Acetone, petroleum ether (60–90 °C), ethyl acetate, *n*-hexane, anhydrous sodium sulfate (pretreated under 650 °C, 2 h), and sodium chloride were used.

Analytical-reagent grade materials were used unless otherwise indicated. Water was glass-distilled before use. Pyrethroid (Analytica, Sigma) stock standard solution (200 $\mu g\ ml^{-1}$ in *n*-hexane) was stored in the dark at 4 °C. Working standard solutions were prepared daily.

Florisil (60–100 mesh; Floridin, Pittsburgh, PA, USA) was heated 24 h at 130 °C and brought to 3% moisture before use. Silica gel (100–200 mesh) and aluminium oxide (reagent powder) were used as contrasts.

2.3. Extraction

A 10.0-g sample of ground tobacco (60–80 mesh) or Cambridge filter discs after collecting the cigarette smoke were extracted and ultrasonicated for 30 min in a flask with 100 ml of acetone added. The macerate was filtered with suction using a Bücher funnel and Whatman No. 1 paper. After filtration through filter paper, the residue was re-extracted with 50 ml acetone for 10 min. The extract flask was rinsed with 20 ml acetone which was added to the funnel after most of the initial filtrate had been collected. The filtrate was completely transferred to a

separatory funnel with acetone rinsing. After 25 ml of petroleum ether was added, the mixture was diluted with saturated sodium chloride (10 ml) and sodium sulfate (2%, 100 ml). Repeating this procedure, the mixture was extracted four times with petroleum ether (25 ml) which was then combined and transferred to a storage bottle. The petroleum ether extract was added with anhydrous sodium sulfate to remove H_2O, which was then stored at freezer temperature until analysis.

2.4. Clean-up

The petroleum ether extract was concentrated in a rotary evaporator at a low pressure at 40 °C. The extract was finally made up to 2 ml and added to the liquid–solid chromatography column. The column was eluted with ten fractions (10 ml each) of a mixture of petroleum ether–ethyl acetate (20:80, v/v), discarding the first three fractions and the last two fractions, and collecting the other ones. The collected fractions were combined and the resulting elute concentrated to 1 ml with a rotary evaporator. The diluting solvent was used as a blank to correct instrumental variations in the quantitative analysis. The injected volume was 1 μl.

2.5. Smoke collection

The cigarette sample was divided into four portions. Of these, three portions were spiked with a known volume of pyrethroid standard solutions; the fourth being the un-spiked control. The cigarettes all had a total length of 85 mm, with a 20-mm filter and no ventilation. They were selected according to a specific value of weight and pressure drop before experiment. They were smoked according to the Coresta Standard Method No. 10 [19], Chopra et al. [20] and the Federal Trade Commission (FTC) [21], i.e. puff duration: 2 s; puff frequency: 1 puff/min; puff volume: 35 ml; butt length: 23 mm. The smoke was collected on a 92-mm diameter Cambridge filter disc. Because of the very low quantities of pyrethroids in cigarette smoke, 60 cigarettes had to be smoked to determine those pyrethroids. After five cigarettes were smoked, the old Cambridge filter pad was replaced by a new filter pad. After all 60 cigarettes had been smoked, all the Cambridge filter pads were collected and extracted with the method described above.

2.6. Capillary GC analysis

The gas-chromatographic conditions were as follows. Injector temperature was 280 °C. Oven temperature was isothermal at 215 °C for 18 min followed by temperature programming to 220 °C at 5 °C min^{-1}. The final temperature was maintained for 18 min. The split ratio was 1:20. 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 length was 30 m, with a 0.25-mm internal diameter and a 0.25-μm liquid film thickness of SE-54 stationary phase (HP-5, Hewlett-Packard, USA). 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 analysis was carried out by comparing the retention time with standards. Quantitative data of pyrethroid residues were obtained by the external method using standards as reference substances, without considering calibration factors (i.e. $F=1.00$ for all compounds).

3. Results and discussion

3.1. Extraction and clean-up

The non-specific nature of the electron capture detector introduced the requirement that a methodology be developed to provide interference-free tobacco and cigarette smoke condense extracts. The acetone maceration of the tobacco and cigarette smoke condensate was not efficient in extracting the pyrethroids. Re-extraction with petroleum ether and quantitative analysis of pyrethroids with clean-up were necessary for the residue analyses.

Florisil at 1.5, 3, and 6% moisture was tested. Suitable amounts of standards were applied directly to the columns in hexane. 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 analyzed. Not all combinations were tried with all

adsorbents. Preliminary results indicated that the Florisil with 3% moisture gave the best recoveries with ethyl acetate–petroleum ether (80:20, v/v). All the pyrethroids were eluted in fractions 4–7 from the Florisil column. Florisil (5, 10, 15, and 20 g) was used in this investigation, and the result showed that 15 g Florisil with 100 ml petroleum ether–ethyl acetate (80:20, v/v) was sufficient for tobacco and cigarette smoke condensate analysis. In a similar manner, the elution patterns of silica gel and aluminum oxide was also examined. The chromatogram of samples fortified by pyrethroid standards eluted with silica gel, aluminum oxide and Florisil was shown in Fig. 1A–C, respectively. A decrease in the number of interfering peaks in GC–ECD gas chromatograms was observed in the Florisil column chromatography clean-up method. This may be related to the characteristics of silica gel, aluminum oxide and Florisil. So Florisil column chromatography clean-up was performed in this investigation.

3.2. GC analysis

Optimal conditions for the determination of pyrethroid was investigated. A gas chromatogram of a pyrethroid-fortified sample is shown in Fig. 1C. Retention times of pesticides investigated here (mean of three trials) are indicated in Table 1. The numbers of peaks in the figure corresponds to the following list: fenpropathrin, cyhalothrin, cypermethrin, fenvalerate and deltamethrin. Compared with traditional pesticide GC determination [14], it was clear that the system shows chromatograms with low drift and low noise, gives a higher sensitivity for the determination of these pyrethroids. This might be attributed to the better resolution of the hydrogen gas and the more effectiveness of Florisil clean-up used in this investigation.

The GLC rate theory of van Deemter can be expressed by the simplified equation for average theoretical plate height. The carrier gas determined the gas phase diffusivity of solute and, therefore, the magnitude of the B and C_g terms in Eqs. (1) and (2). It could be shown that the optimum velocity was proportional to D_g (diffusivity of gas phase), where the C_l term is negligible. So the relative optimum velocities for N_2:He:H_2 would be 1:2.5:3.5 [22]. It could also be shown that H was independent of D_g, so that with H_2, chromatography could be completed in about one-third the time required with N_2 at the same efficiency. Comparison of H_2 with N_2 as carrier gas is also shown in Figs. 1C and 2. The 30-m capillary column system gave the best resolution for all compounds. All five pyrethroids could be determined on this system.

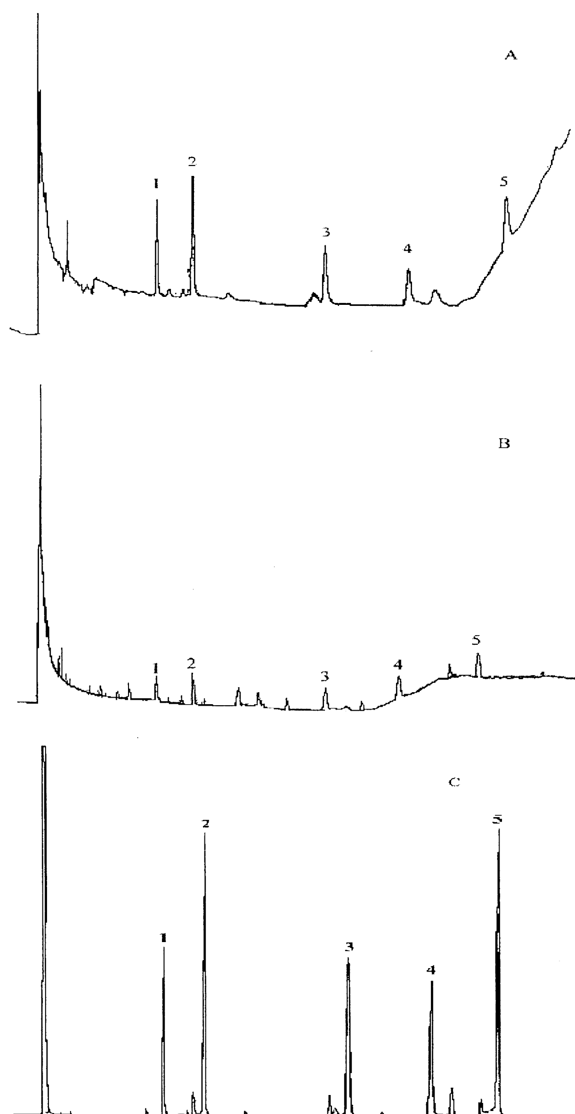

Fig. 1. Gas chromatogram of silica gel (A), aluminum oxide (B) and Florisil (C) clean-up of pyrethroid-fortified tobacco samples (0.1 μg g^{-1}). The identified peaks are shown in Table 2.

Table 1
Linear equations and determination limits (DL)

Pesticide	t_R (min)	Linear equation	Regression coefficient	DL
Fenpropathrin	7.19	$y = 82270x - 5257$	0.997	0.060
Cyhalothrin	9.12	$y = 15057x - 11308$	0.997	0.075
Cypermethrin	16.1	$y = 113330x - 8058$	0.993	0.072
Fenvalerate	20.1	$y = 105432x - 8596$	0.995	0.082
Deltamethrin	23.3	$y = 143912x - 22915$	0.989	0.014

$$H = A + \frac{B}{u} + Cu \quad (1)$$

$$Cu = (C_l + C_g)\, u \quad (2)$$

C_l = liquid⇔gas (through the liquid)

$$C_g = \begin{cases} C_1 = \text{gas⇔liquid (through interparticle and pore space)} \\ C_2 = \text{gas⇔gas (to different velocity streams)} \end{cases}$$

All pyrethroid residues examined were quantitatively analyzed under the above GC conditions. Detector responses were linear; their ranges are 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, are shown in Table 1.

3.3. Method check-up

The method was checked by using fortified samples of tobacco. These were prepared by adding known volumes of mixed pyrethroid standard solution in hexane to 10-g suitable portions of ground tobacco shown not to contain residues of the pyrethroids (as shown in Table 2). The flask containing the fortified tobacco was shaken to ensure even distribution of the pyrethroids 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 the pyrethroids to be absorbed on the tobacco and correspond more closely to a field-treated commercial tobaccos. The samples were then extracted and determined as described in Section 2. Recoveries of pyrethroids are 76.2–111.2% and the standard deviation of reproducibility (RSD) are 4.12–13.3%. Finally, as an example of routine determination, a chromatogram obtained by adding known volumes of mixed pyrethroid standard solution to tobacco samples is shown in Fig. 1C. The overall efficiency and variability was in the range reported for many residue analyses.

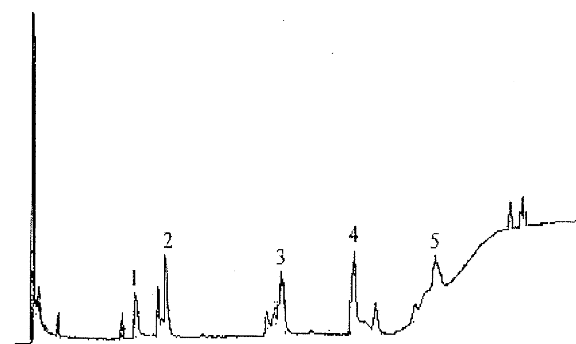

Fig. 2. Chromatogram of fortified tobacco sample (0.1 μg g^{-1}) using nitrogen as carrier gas. The identified peaks are shown in Table 2.

Table 2
Result of recovery and its relative standard derivation

Pesticide	Added (μg g^{-1})	Recovery (%)	RSD ($n=5$) (%)
Fenpropathrin	0.2	86.3	8.15
	2.0	111.2	4.12
Cyhalothrin	0.01	76.2	13.3
	0.1	99.4	10.4
Cypermethrin	0.2	81.2	9.89
	2.0	96.3	7.10
Fenvalerate	0.2	83.6	11.2
	2.0	97.2	6.09
Deltamethrin	0.01	80.3	12.2
	0.1	98.3	9.76

Table 3
Transfer rate (R) of pyrethroids from tobacco into cigarette smoke

Pesticide	Added (M_A, μg)	Blank (M_B, μg)	Transfer rate (R, %)	RSD ($n=5$) (s_r, %)
Fenpropathrin	5.37	0.19	15.5	7.51
Cyhalothrin	4.72	–	5.20	18.4
Cypermethrin	5.03	0.10	1.51	10.1
Fenvalerate	4.11	–	1.72	2.34
Deltamethrin	5.35	–	2.64	13.8

M_A means amount of pyrethroid in cigarettes spiked with standard. M_B means amount of pyrethroid in cigarettes without spiked standard.

3.4. Pyrethroid residues in cigarette smoke

Five batches of 60 cigarettes of spiked and unspiked tobacco were smoked as described above and their smoke condensates were combined and extracted, cleaned-up and determined. The transfer rate of pyrethroids from tobacco into cigarette smoke was from 1.5 to 16%, and the standard deviation of reproducibility (RSD) was from 2.3 to 18% (as shown in Table 3). The present transfer of pyrethroids into the smoke of field-treated tobacco might be much less than that from artificially spiked tobacco (Table 3). This result seemed reasonable because the systematically incorporated residues in field-treated sample are less subject to volatilization than the surface residues applied with an atomizer to shredded tobacco. Therefore, during the smoking process, the incorporated residues appear to be subject to greater pyrolytic degradation than surface residues. Fenpropathrin appeared to be transferred to the smoke more readily than cypermethrin. The studies with spiked tobacco showed that the amount of fenpropathrin transferred to the smoke was more than ten times the amount of cypermethrin transferred from tobacco with comparable residue levels. The results confirmed the hazard the pesticide-use might pose to the smoker. This is important in controlling the limit of pesticide residue in tobacco and the estimation of transfer rate from tobacco into cigarette smoke. Therefore, the commercial use of pyrethroids on tobacco must also consider their detectable residues in tobacco smoke.

3.5. Pyrethroid residues in tobacco

The method was found to be effective for simultaneous determination of the five pyrethroid residues. The five pyrethroid pesticides examined in this study were monitored by the proposed method of multi-residue analysis in nine tobacco samples from three different area: Guizhou, Yunnan and Henan. The results are shown in Table 4. The determination limit was 0.01 $\mu g\ g^{-1}$ for pyrethroid pesticides by the proposed method. The results identify the different types of pyrethroid and the amounts used to treat the tobaccos. Therefore, this method could be extended for commercial residue-screening of tobacco imports and exports.

4. Conclusion

Effective procedures have been developed for the extraction of five pyrethroids from tobaccos and cigarette smoke, and the removal of tobacco extracts by Florisil column chromatography to permit analy-

Table 4
Content of five synthetic pyrethroids in tobacco from three different provinces in China ($\mu g\ g^{-1}$)

Pesticide	Guizhou			Yunnan			Henan		
	98C3L	98X3F	96B3L	97B4F	98C2L	96C1L	97B2F	98C3F	96X2F
Fenpropathrin	–	–	–	0.12	0.21	0.19	0.68	0.10	0.21
Cyhalothrin	–	–	–	–	–	–	–	–	–
Cypermethrin	0.63	–	0.12	0.06	–	0.07	–	–	–
Fenvalerate	–	–	–	–	–	–	–	0.11	0.28
Deltamethrin	0.46	0.09	–	–	–	–	–	–	–

Guizhou, Yunan and Henan are the places where tobacco (*Nicotina tobaccum*) is grown. 98C3L, 98X3F, 96B3L, etc. are the grades of tobacco.

sis of the pyrethroids at residue levels by GC–ECD. These procedures represent our current ''best'' solution to a 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 other pyrethroid pesticides.

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