

Determination of Pyrethroid Pesticide Residues in Vegetables by Solvent Sublation Followed by High-Performance Liquid Chromatography

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Abstract

A novel method is developed for separating and enriching pyrethroid pesticides from vegetables by solvent sublation, and determination of the pyrethroids is performed by high-performance liquid chromatography (HPLC). The effects of organic solvent, pH of the solution, nitrogen flow rate, and sublation time on the sublation efficiency of pyrethroids are investigated in detail, and the optimal conditions of the solvent sublation are selected. The floated product of vegetables in the optimal conditions is determined by HPLC. The limit of detection values range from 1.4 $\mu\text{g}/\text{kg}$ (for bifenthrin) to 4.2 $\mu\text{g}/\text{kg}$ (for fenpropathin). The recoveries of spiked vegetable samples are from 85.7% to 110.4%, and relative standard deviation values are from 1.70% to 6.19%. The results are satisfactory.

Introduction

Pyrethroids are organic synthetic insecticides that are widely used for the protection of crops and food storage against insects and acarids (1). Nevertheless, pyrethroid residues in vegetables after application to the crops pose risks to human health and other species. Therefore, monitoring of pyrethroid residue levels in vegetables is of particular concern for human health (2). There have been a variety of approaches developed in recent years to quantify pyrethroids in environmental and biological samples. Immunoassays have been developed to rapidly detect trace levels of pyrethroids in environmental and food samples (3,4). A pressurized isocratic capillary electrochromatography method has been developed to separate and determine pyrethroid pesticide residues in vegetables (5). López-López et al. (6) determined pyrethroids in vegetables by high-performance liquid chromatography (HPLC) using continuous on-line post-elution photoradiation with fluorescence detection. A more rapid and sensitive HPLC method for analyzing the pyrethroid insecticide in rat plasma and tissues has been developed and validated according to the U.S. (7).

Solvent sublation was originally introduced by Sebba (8) as an auxiliary method to ion flotation. It is a kind of adsorptive bubble separation technique, in which the hydrophobic compounds in

water are adsorbed on the bubble surfaces of an ascending gas stream and then collected in an immiscible liquid layer placed on top of the water column. This method, with its advantages of simultaneous separation and enrichment, has recently attracted much attention in the fields of environmental analysis and wastewater treatment (9–15).

Solvent sublation has practical and theoretical advantages over other extraction methods (such as solvent extraction and solid-phase extraction). Firstly, the solvent sublation process is not limited by the equilibrium constant, so the recovery of trace substances can eventually reach 100%. Secondly, in solvent sublation, the possibility of easy handling of large volumes of aqueous samples exists, whereby the enrichment factors can easily exceed ratios of 100:1, thus making the techniques of great potential interest for the analysis of natural, residual, and marine waters for trace substances.

In this paper, fenpropathin, deltamethrin, and bifenthrin were selected as model compounds. The structures of these pyrethroids have hydrophobic groups; they can be adsorbed on the bubble surfaces of an ascending gas stream and then dissolved in some kinds of organic solvent placed on the surface of the sample solution. Therefore, they are suitable for solvent sublation. The effects of organic solvent, pH of the solution, nitrogen flow rate, and sublation time on the efficiency of pyrethroid solvent sublation were investigated in detail. The floated product in the optimal conditions was measured by HPLC. The proposed method was applied to vegetable samples such as lettuce, celery, cabbage, and swamp cabbage with good results.

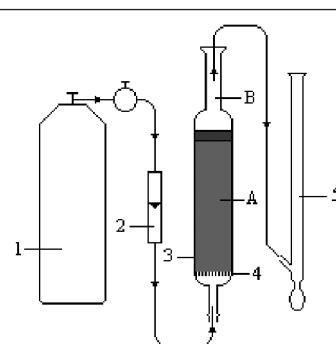


Figure 1. Solvent sublation apparatus: 1, Nitrogen cylinder; 2, pin-type flow meter; 3, flotation cell; 4, sintered glass disk (G4 porosity); 5, soap-bubble flow-meter.

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Experimental

Instrumentation

A Mettler Toledo 320-S pH meter (Mettler, Switzerland) was used to determine the pH of the solution. A U-3010 UV-vis spectrophotometer (Hitachi, Japan) was used to acquire the absorption spectrum. A KQ-100A supersonic wave purifier (Kunshan, China) and an AB204-N electron balance (Mettler) were used. An Agilent series 1100 HPLC (Palo Alto, CA) equipped with UV-vis detector was used for qualitative and quantitative analysis of the floated product. An Eclipse XDB-C18 chromatographic column (5 μm , 150 mm \times 4.6 mm) was used. The mobile phase composition was 85:15 (v/v) acetonitrile–water, with a flow rate of 1.0 mL/min. It was filtered prior to use through a membrane filter (0.45 μm). Detection wavelength was 215 nm. The injection volumes of samples and standard solutions were all 10 μL . The system was controlled by a computer and Chemstation software from Agilent. All chromatographic analyses were performed at room temperature.

Figure 1 shows the solvent sublation apparatus. The apparatus consists of a glass cylinder equipped with a sintered glass disk (G4 porosity) at the bottom to generate small bubbles. The disk is connected to an N₂ gas cylinder equipped with a pressure regulator by a fine pressure needle valve for controlling the gas flow. For accurately measuring the gas flow rate through the cylinder, a soap-bubble flow-meter was inserted into the cylinder. The cylinder, with an inner diameter of 4.5 cm of part A and a capacity of 400 mL, is designed for flotation. Sample solution is transferred to the flotation cell, then a suitable organic solvent is added to the top of the sample solution with a volumetric pipet. After flotation, deionized water is added to the top of the cylinder to make the organic phase rise to part B, which has an inner diameter of 2.0 cm and a capacity of 15 mL, from which it can be easily removed. Finally, when the layers have separated, the organic phase is taken into a volumetric flask with a dropping pipet, and marked with the organic solvent.

Reagents and samples

Toluene, *n*-octanol, *n*-hexane, acetone, isoamyl alcohol, hydrochloric acid, and sodium hydroxide (Beijing Chemical Reagent Factory, China) were all of analytical-reagent grade. Methanol (Tianjing Xihua Special Reagent Factory, China) was chromatographic grade. The individual pyrethroid standards

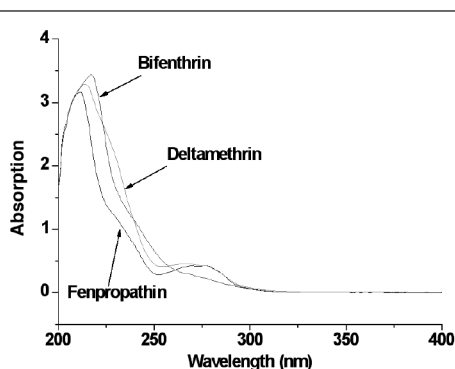


Figure 2. Absorption spectra of pyrethroids.

including fenpropathin (purity > 99.9%), deltamethrin (purity > 99.7%), and bifenthrin (purity > 99.6%) were obtained from National Research Center for CRM (Beijing, China). Standard stock solutions of pyrethroids (1.0 mg/mL) were prepared by exactly weighing and dissolving corresponding compounds in methanol, and storing in a refrigerator at 4°C. Standard solutions (0.33 $\mu\text{g/mL}$) were freshly prepared by diluting the standard stock solutions with deionized water before each use. The vegetable samples, including lettuce, celery, cabbage, and swamp cabbage, were purchased from the market nearby.

Procedure

A 75 g portion of vegetables was weighed, cut up, and placed into a 300-mL beaker. Deionized water (135 mL) and 15 mL acetone were added, and the beaker was placed into a supersonic wave purifier for 25 min at 55 kHz. In this process, the pyrethroid residues in vegetables were extracted to the mixed solvent of deionized water and acetone. The contents of the beaker were filtrated and then the filtrate was collected. The same process was repeated once again. Then the two filtrates were combined and the pH was adjusted to 3 with 0.1 mol/L hydrochloric acid solution. This solution was transferred to a flotation cell (as shown in Figure 1), then 10.00 mL of *n*-hexane were added in, and pyrethroids were floated by bubbling nitrogen

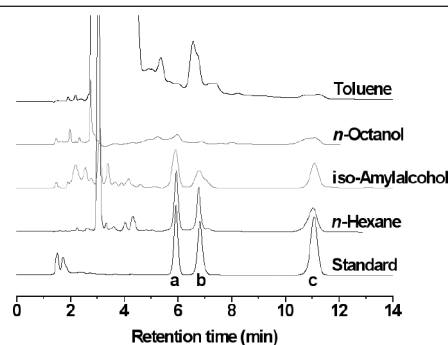


Figure 3. Effect of sublation solvents. Fenpropathin (a); deltamethrin (b); bifenthrin (c).

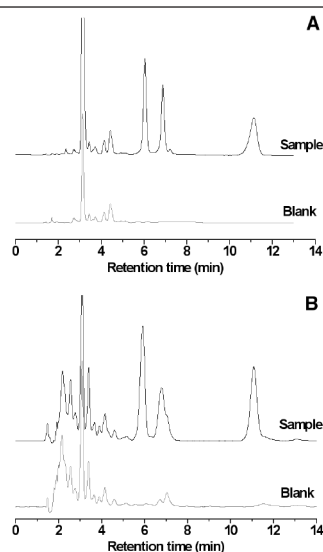


Figure 4. The effect comparison between *n*-hexane and isoamyl alcohol. *n*-Hexane as sublation solvent (A); isoamyl alcohol as sublation solvent (B).

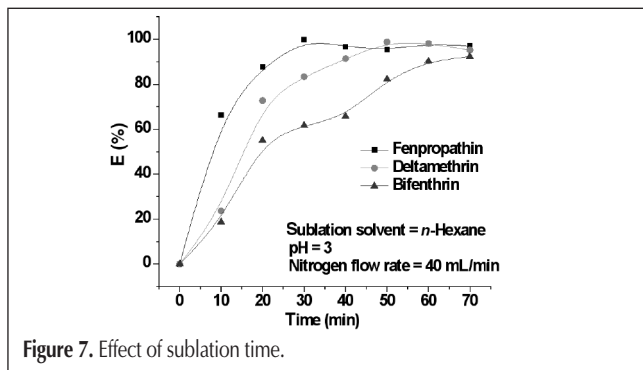
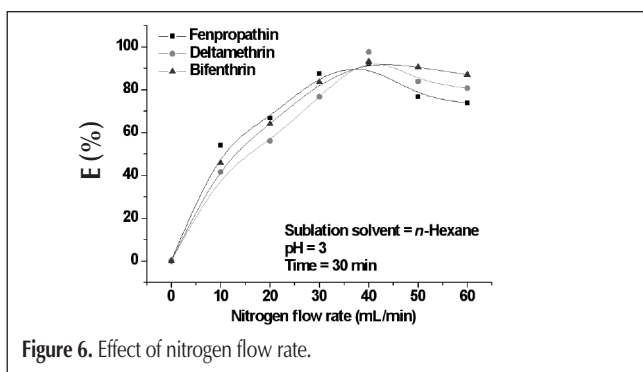
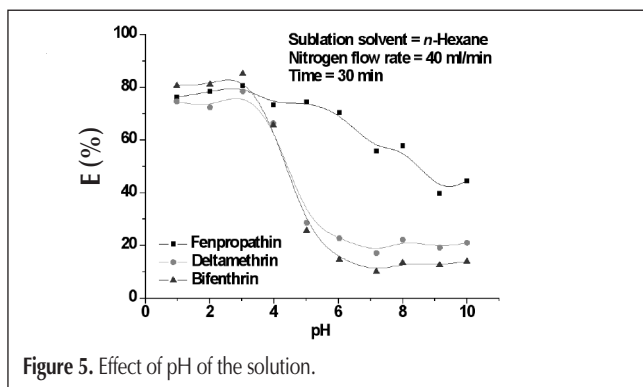
gas at the flow rate of 40 mL/min from the bottom of the cell for 60 min, and extracted into 10.00 mL of *n*-hexane on the surface of the sample solution. The *n*-hexane phase was then transferred to a 10-mL volumetric flask and marked with *n*-hexane. Finally, the pyrethroids in the *n*-hexane phase were determined by HPLC.

Results and Discussion

Optimization of the solvent sublation parameters

In this work, the sublation efficiency of pyrethroids in the standard solutions was used for optimizing the parameters affecting the pyrethroid solvent sublation. The sublation efficiency of pyrethroids can be calculated by use of the following equation.

$$E = C_i / C_0 \times 100\% \quad \text{Eq. 1}$$



In the formula, E is the sublation efficiency, C_i is the concentration of the *n*-hexane phase after flotation, and C_0 is the concentration of *n*-hexane phase that supposing 100% of the pyrethroids in the aqueous phase was floated into. The concentration of pyrethroids in the *n*-hexane phase was determined by HPLC using an external standard method.

Absorption spectra

As shown in Figure 2, the maximum absorbances in *n*-hexane were at 212 nm for fenpropathin, 215 nm for deltamethrin, and 220 nm for bifenthrin, respectively. In this work, 215 nm was used as the measurement wavelength of the UV-vis detector.

Effect of sublation solvent

There are some restrictions in choosing a sublation solvent. That is, the solvent should have high affinity with the pyrethroids, be lighter than a sample solution, and have a low volatility. The influence of organic solvents such as *n*-hexane, *n*-octanol, isoamyl alcohol, and toluene were investigated for effective sublation. The experimental results (Figure 3) showed that of all the solvents the best solvent was *n*-hexane, and the next was isoamyl alcohol.

As shown in Figure 4, the background interference of *n*-hexane as sublation solvent is less than that of isoamyl alcohol as sublation solvent. Furthermore, the sublation efficiency of fenpropathin, deltamethrin, and bifenthrin were 82.10%, 61.60%, and 56.01% using *n*-hexane as sublation solvent, and 74.61%, 33.40%, and 56.33% using isoamyl alcohol as sublation solvent. So *n*-hexane was selected as the pyrethroid sublation solvent.

Effect of pH

An experiment was performed by changing the pH from 1 to 10 by adding 0.1 mol/L hydrochloric acid solution or 0.1 mol/L sodium hydroxide solution. The pyrethroids in the standard solutions were floated into 10.00 mL of *n*-hexane at the flow rate of 40 mL/min for 30 min at each pH. The experimental results (Figure 5) showed that the sublation efficiency of pyrethroids increased slightly with the increase of pH in the pH range of 1–3, and at pH 3, the maximum sublation efficiency appeared. However, at pH values higher than 3, the sublation efficiency decreased. Therefore, pH 3 could be selected as an optimal pH for the efficient sublation.

Effect of nitrogen flow-rate

As is well known, the bubbling of nitrogen is very important in solvent sublation because the bubbles can float the hydrophobic analytes to the surface of the solution. As the bubbles rise

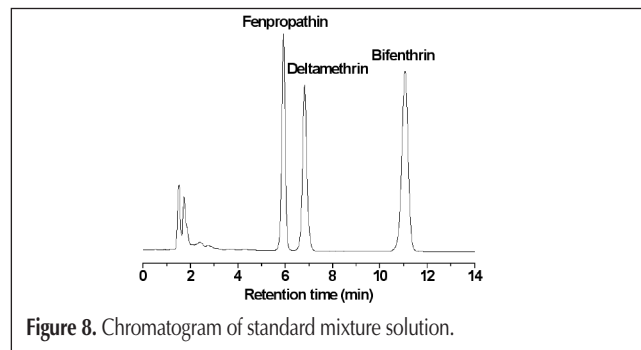


Table I. The Comparison Results Between Solvent Sublation and Solvent Extraction (n = 5)*

Method	Pesticide	Recoveries (%)	Enrichment factors	$V_{\text{aqueous}}:V_{\text{organic}}$
Solvent sublation	Fenpropathin	97.29	29.19	30:1
	Deltamethrin	95.68	28.70	
	Bifenthrin	97.03	29.11	
Solvent extraction	Fenpropathin	95.44	0.95	1:1
	Deltamethrin	95.86	0.96	
	Bifenthrin	96.05	0.96	

* The concentration of standard solution: fenpropathin, 70 µg/L; deltamethrin, 68.67 µg/L; and bifenthrin, 72.67 µg/L; respectively.

Table II. Determination Results of Real Samples by Solvent Sublation Followed by High-Performance Liquid Chromatography*

Sample	Pesticide	Content in sample (mg/kg)	Added (mg/kg)	Found (mg/kg)	Recovered (mg/kg)	Recoveries (%)	RSD (%)	
Lettuce	Fenpropathin	-	0.028	0.024	0.024	85.7	6.19	
			0.112	0.105	0.105	93.8		
			0.224	0.217	0.217	96.7		
	Deltamethrin		0.027	0.026	0.026	97.9		2.59
			0.116	0.108	0.108	93.1		
			0.219	0.207	0.207	94.5		
	Bifenthrine		0.029	0.030	0.030	103.4		3.56
			0.116	0.114	0.114	98.3		
			0.233	0.225	0.225	96.6		
Celery	Fenpropathin	-	0.028	0.025	0.025	89.3	5.01	
			0.112	0.105	0.105	93.8		
			0.224	0.221	0.221	98.7		
	Deltamethrin		0.027	0.026	0.026	96.3		3.29
			0.110	0.106	0.106	96.4		
			0.220	0.227	0.227	103.2		
	Bifenthrine		0.029	0.028	0.028	96.6		1.70
			0.116	0.109	0.109	94.0		
			0.233	0.226	0.226	97.0		
Cabbage	Fenpropathin	-	0.028	0.024	0.024	85.7	5.87	
			0.112	0.102	0.102	91.1		
			0.224	0.216	0.216	96.4		
	Deltamethrin		0.027	0.024	0.024	89.5		2.88
			0.110	0.104	0.104	94.5		
			0.220	0.206	0.206	93.6		
	Bifenthrine		0.029	0.032	0.032	110.4		3.81
			0.116	0.126	0.126	108.6		
			0.233	0.239	0.239	102.6		
Swamp cabbage	Fenpropathin	-	0.028	0.025	0.025	89.3	4.53	
			0.112	0.102	0.102	91.1		
			0.224	0.218	0.218	97.3		
	Deltamethrin		0.027	0.025	0.025	92.6		1.72
			0.110	0.103	0.103	93.6		
			0.220	0.199	0.199	90.5		
	Bifenthrine		0.029	0.026	0.026	89.7		1.86
			0.116	0.108	0.108	93.1		
			0.233	0.213	0.213	91.4		

* " - " means "not detected"; the weight of vegetables is wet weight.

through the gas diffuser, the hydrophobic analytes are adsorbed at the gas-liquid interface and then extracted into the organic phase on the surface of the sample solution. Generally, the rate of gas-liquid interfacial area generation can be increased by generating smaller bubbles via a gas diffuser with smaller porosity or by increasing the gas flow rate. However, it is recommended that too-high gas flow rates are to be avoided because of a turbulent mixing at the solvent-aqueous solution interface. Such a mixing can promote the re-dissolution of the hydrophobic analytes in the aqueous phase.

As shown in Figure 6, the sublation efficiency of pyrethroids increased with increasing nitrogen flow rate; however, the maximum efficiency was obtained at 40 mL/min. When the nitrogen flow rate was higher than 40 mL/min, the efficiency decreased slightly. Therefore, the nitrogen flow rate could be fixed at 40 mL/min in all subsequent experiments.

Effect of sublation time

As shown in Figure 7, the sublation efficiency of pyrethroids increased with an increase in sublation time; however, when the sublation time ≥ 60 min, the efficiency reached its highest value and basically remained constant because of the achievement of the thermodynamic equilibrium. So the sublation time could be fixed at 60 min in this work.

Comparison of the solvent sublation with solvent extraction

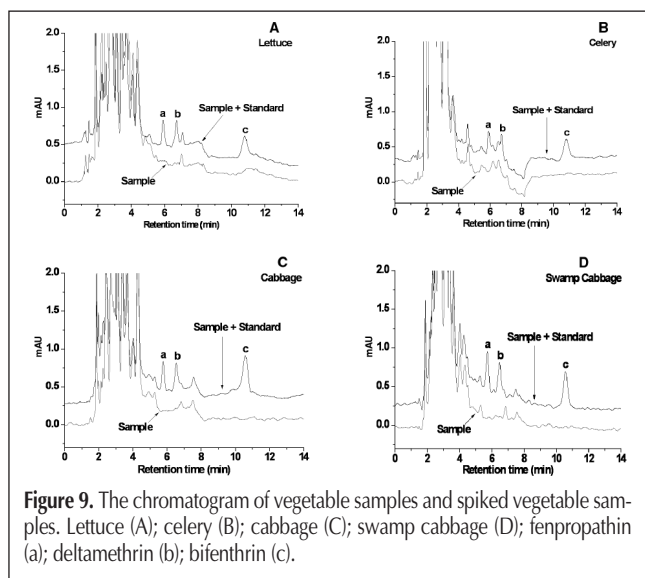
In this work, solvent extraction was used to separate and concentrate fenpropathin, deltamethrin, and bifenthrin from the standard solutions for the purpose of comparison with solvent sublation. The results obtained are summarized in Table I.

From Table I, it can be seen that the efficiency of pyrethroid solvent sublation is better than that of solvent extraction. In addition, the phase stirring process associated with liquid-liquid extraction frequently leads to the formation of undesirable emulsions. In the solvent sublation process, however, emulsion formation is negligible owing to the absence of the phase mixing process, so that the solvent sublation method can offer simplicity and rapidity.

HPLC analysis

Analysis of the floated product

The qualitative and quantitative analysis of the floated product was performed using an Agilent series 1100 HPLC. Under the selected chromatographic conditions (mobile phase: acetonitrile-water 85:15 [v/v]; detection wavelength, 215 nm; flow rate, 1.0 mL/min;



injection volume, 10 μ L), the chromatogram for the mixture of three pyrethroids is shown in Figure 8. It can be seen that three analytes were baseline-separated within 12 min.

Calibration curve and detection limit

Calibration curves for fenpropathin, deltamethrin, and bifenthrin were obtained over the ranges 7.00–70.00, 6.87–68.67, and 7.27–72.67 μ g/L, respectively. The regression equations were $y = 1.08139x - 0.64656$ ($R^2 = 0.9998$) for fenpropathin, $y = 1.10078x + 0.32177$ ($R^2 = 0.9989$) for deltamethrin, and $y = 1.77874x - 1.01049$ ($R^2 = 0.9996$) for bifenthrin, where y is the peak area and x is the concentration (μ g/L). The limit of detection (LOD) values for three pyrethroids were based on the lowest detectable peak that had signal/noise = 3. In this work, LOD values were 4.2 μ g/kg for fenpropathin, 2.4 μ g/kg for deltamethrin, and 1.4 μ g/kg for bifenthrin; it can be seen that the LOD values of the proposed method is lower than that of the common HPLC method (16,17).

Determination of pyrethroid residues in real samples

The optimized experimental conditions were applied to real samples to evaluate the efficiency of the determination of pyrethroids by solvent sublation followed by HPLC. The determination results are shown in Figure 9 and in Table II. None of the three pyrethroids was found in the samples. The recoveries were examined in the samples in which given amounts of analytes were determined by the proposed method. The recoveries obtained were in the range of 85.7–110.4%, and relative standard deviation (RSD) values were in the range of 1.70–6.19%, it follows that the accuracy and precision of the proposed method for determination of fenpropathin, deltamethrin, and bifenthrin in vegetables are satisfactory.

Conclusions

In this research, a novel and effective method for separating and enriching pyrethroids from vegetables by solvent sublation and its determination by HPLC was established. Under the selected optimal conditions, the recoveries ranged from 85.7%

to 110.4%, RSD values from 1.70% to 6.19%, and the LOD values were 4.2 μ g/kg for fenpropathin, 2.4 μ g/kg for deltamethrin, and 1.4 μ g/kg for bifenthrin. The results show that the proposed method can be used to analyze trace pyrethroid pesticide residues in vegetables.

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