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Research Article

ANALYSIS OF FENPROPATHRIN, LAMBDA-CYHALOTHRIN, AND CHLOROTHALONIL IN POTATO AND TOMATO SAMPLES USING GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

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ABSTRACT

Objective: This study aimed to analyze pesticide contents in potato and tomato samples.

Methods: In the present study, we determined the presence of the pesticides fenpropathrin, lambda-cyhalothrin, and chlorothalonil in conventional and organic potatoes and tomatoes using a gas chromatograph equipped with an electron capture detector and validated the associated methods. Acetone-based extraction was performed using the Dutch mini-Luke method with minimal weights and volumes.

Results: Validation tests showed a range of 70–120% and precision of ≤20%, and linearity tests on the three standard pesticides gave r values of ≥0.9990 for all three pesticides. Limit of detection and limit of quantitation values showed high sensitivity, although in vegetable sample analyses, none of the three pesticides were detected.

Conclusion: Our data show that the chosen method for analysis of the pesticides fenpropathrin, lambda-cyhalothrin, and chlorothalonil in potatoes and tomatoes is valid and that the marketed potatoes and tomatoes meet the SNI 7313: 2008 standard for "Maximum Limits of Pesticide Residues on Agricultural Products" and the associated Japanese standards.

Keywords: Gas chromatography, Electron capture detector, Organic vegetable, Non-organic vegetable, Fenpropathrin, Lambda-cyhalothrin, chlorothalonil, Dutch mini-Luke.

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INTRODUCTION

Pesticides are used to eliminate pests so that agricultural harvests can be optimal and are also used to preserve woods and forestry products. Hence, human pesticide use has grown and developed considerably, and formerly used natural pesticides have been replaced with artificial agents [1].

Due to toxic effects in humans, animals, and the environment, bans have been placed on the use of several pesticides, including organochlorine agents such as aldrin, eldrin, DDT, and BHC [2]. Organochlorines are known persistent organic pollutants [3] that bioaccumulate and disperse into food chains. The fat solubility of these organochlorine pesticides is an important contributor to chronic toxicity. Moreover, DDT affects axonal transmission by decreasing sodium intake and inhibiting egress of potassium. Cyclodiene and hexachlorocyclohexane also inhibit chloride uptake in response to gamma-aminobutyric acid, which is a central nervous system mediator. As a consequence, these agents cause neuron hyperactivity, and death can occur within 24–72 h due to consequent breathing disturbances [4].

Despite their chronic toxic effects in humans and proven persistence in the environment, many organochlorines, such as chlorothalonil, have been used in Indonesia [5].

In addition to organochlorine, organophosphates, carbamates, and pyrethroids are used in Indonesia. Pyrethroid is a derivative of a molecule from chrysan flowers [6] and is classified into two types on the basis of the presence or absence of cyan (C≡N). Addition of cyan to this pesticide increases its insecticidal activity [7]. Cyan groups, including in fenpropathrin and lambda-cyhalothrin, can cause reversible paresthetic fever, numbness, tickling sensations of the skin, and nervous system perturbations [6]. Hence, evaluations of pesticide concentrations are crucial for food products. Several studies show health effects of pyrethroid residues in contaminated fruit and vegetables, and chronic consumption has a high potential to adversely affect humans [7].

Organic vegetables have become increasingly popular in recent years, on the basis of the assumptions of higher nutrition and the absence of toxic chemicals [8]. On the basis of a study by Yu and Yang in Singapore, 1 in 10 organic vegetables was not free of pesticide [9-11]. Hence, since fenpropathrin, lambda-cyhalothrin, and chlorothalonil are used in tomato and potato commodities [5], we tested samples of these vegetables.

Concentrations of these three pesticides were analyzed using a high activity liquid chromatography method with gas chromatography (GC) and a mass spectrometer (MS) detector and electron capture detector (ECD). The data herein show high sensitivity and selectivity of the ECD.

MATERIALS AND METHODS

Materials

Non-organic and organic potatoes and tomatoes of various brands were purchased in Depok, Jakarta, and Tangerang. The standard pesticides fenpropathrin, lambda-cyhalothrin, and chlorothalonil were purchased as 200-ppm solutions in acetone from National Plant Quality Assay Laboratory in Pasar Minggu, Jakarta, and were stored at 20°C in a refrigerator. Acetone pure absolute (p.a.; Merck, US), sodium sulfate anhydrate p.a. (Merck, US), petroleum ether p.a. (Merck, US Canada), dichloromethane p.a. (Merck, US), isooctane p.a. (Merck, US), and toluene p.a. (Merck, US) were purchased from respective suppliers.

Analytical instrumentation

Analyses were performed using a Shimadzu model GC-17A GC with an ECD, a 30-m capillary column of 0.25 mm in diameter and 0.25 μm in

film thickness, nitrogen carrier gas, GC solution, a 5.0-µL microsyringe (Hamilton Co., Nevada, USA), scales, vortex, centrifuge, blender, evaporator, and glass tools.

Procedures

Preparation of solutions

Standard primary solutions were prepared by evaporating standard pesticide (200 ppm) solutions of up to 1 ml under a flow of nitrogen gas in a 60°C evaporator over 10 min. Pesticide residues were then dissolved in 10-ml aliquots of isooctane-toluene (9:1) to produce solutions of 20 ppm as described by Lozano *et al*. [12,13].

The calibration curve of fenpropathrin standard liquid was prepared by placing 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, and 4.5-ml aliquots of 100-ppm solutions into six 10-ml glass measuring flasks and filling to volume with isooctane-toluene (9:1). Standard fenpropathrin concentrations were of 20, 25, 30, 35, 40, and 45 ppb. Using 100-ppb cyhalothrin standard solution, we prepared standard solutions of 15, 20, 25, 30, and 40 ppb. Similarly, from 100-ppb cyhalothrin solution, we prepared solutions at 5, 10, 15, 20, 25, and 30 ppb.

Analysis conditions

Analyses were performed with an injection volume of 1 µL and an injector and detector temperature of 300°C using nitrogen as the carrier gas at 1 ml/min. The oven temperature program was 80°C for 1 min, followed by increases to 180°C at 25°C/min and then to 280°C at 8°C/min and finally to 300°C at 30°C/min. The temperature was then maintained at 300°C for 3.17 min. The total time of analysis was about 22 min (Lozano *et al*., 2016).

Method validation

Linearity tests were performed by injecting six standard solutions of various concentrations. Data were then analyzed using linear regression of concentration versus area, and corresponding r values were generated. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the calibration curve [14].

Precision tests and extractions were conducted as described by Lozano *et al*. at three concentrations [12,13]. Simulations were performed with organic vegetables as matrix, and pesticide-free status was confirmed. The requirements of this study were f70%–120% and percentage VC <20% [11].

Standard solutions of 200 ppm in acetone were diluted to 100 ppb and were added to the matrix and then extracted as described previously [12,13]. Fenpropathrin was diluted in acetone to 80, 140, and 180 ppb. Subsequently, 6.0-g samples were weighed into 18 different centrifuge tubes and 5-ml aliquots of 20-ppb fenpropathrin solution were added to the first six tubes, 5-ml aliquots of 35-ppb fenpropathrin were added to glass tubes 7–12, 5-ml aliquots of 45-ppb fenpropathrin in acetone were added to tubes 13–18, and all tubes were shaken and then left standing for 5 min. 6 g of anhydrate Na2SO4 and 3 ml of acetone were then added, and all tubes were centrifuged for 30 s at 1500 rpm (extraction stage). Thereafter, 8-ml aliquots of petroleum ether and 4-ml aliquots of dichloromethane were added, and the tubes were centrifuged again for 30 s at 1500 rpm (partition stage). After centrifuging at 3300 rpm for 3 min, up to 3 ml of the upper layers were taken and evaporated in a water boiler with increasing temperature from 45 C until extracts were dry. Residues were diluted in 0.9 ml of isooctane-toluene (9:1) and were analyzed under the described conditions. The resulting fenpropathrin solutions had concentrations of 20, 35, and 45 ppb, and 1-, 10-, and 40-ppb lambda-cyhalothrin, and 5-, 20-, and 30-ppb chlorothalonil solutions were processed as described above.

Sample preparation

1 km samples of conventional and organic potatoes and tomatoes of differing brands were purchased in Depok, Jakarta, and Tangerang. Extraction procedures were performed according to the study by Lozano *et al*. but with a volume: weight ratio of 2:5 [12,13]. Vegetables were cut and blended, and 0.1-g samples were placed in centrifuge tubes with 8 ml of acetone and 6 g of anhydrate Na2SO4. After centrifuging at 1500 rpm for 30 s (extraction stage), 8-ml aliquots of petroleum ether and 4-ml aliquots of dichloromethane were added and the tubes were centrifuged again for 30 s at 1500 rpm (partition stage) and then at 3300 rpm for 3 min. Up to 3-ml upper layers were then taken and evaporated in a water bath with increasing temperature from 45°C to 63°C until residues were dry. Residues were finally dissolved in 0.9-ml aliquots of isooctane-toluene (9:1) and were analyzed as described above.

Establishment of samples

Qualitative and quantitative analyses were performed with 1-µL samples in triplicate using the described analysis conditions.

RESULTS AND DISCUSSION

Condition analysis

Analyses were performed as described by Lozano *et al*. with slight modifications [13]. Briefly, the oven temperature program was 80°C for 1 min, followed by increasing temperature to 180°C at 25°C/min, to 280°C at 8°C/min, and then to 300°C at 30°C/min, which was maintained for 3.17 min. The total analysis time was about 22 min. Comparisons with the analysis conditions described by Lozano *et al*. are listed in Table 1 [12,13].

Establishment of retention times

After injecting fenpropathrin, lambda-cyhalothrin, and chlorothalonil at 20 ppm, retention times were 18.363, 19.278, and 10.851 min, respectively.

Validation of analysis methods

Linearity tests

Linearity was tested using fenpropathrin at 20, 25, 30, 35, 40, and 45 ppb. Regression analysis of concentration versus area gave the linear equation y=583.57x−4418.2 with an r value of 0.9994. Linearity tests for lambda-cyhalothrin were performed at 10, 15, 20, 25, 30, and 40 ppb and gave the equation y=1879.4x+12787 with an r value of 0.9992. For chlorothalonil at 5, 10, 15, 20, 25, and 30 ppb, the linear equation y=1061.7x−177.8 was generated with an r value of 0.9993.

Table 1: Differences between analysis conditions between Lozano *et al*. and the present study

Notes	Lozano *et al.*	This study
Tools	Bruker 436 GC	Shimadzu GC-17A
Detector	MS	ECD
Columns	Capillary columns 30 m in length and 0.25 mm in diameter, 0.25-µm film thickness with VF-5 idle phase	Capillary columns 30 m in length and 0.25 mm in diameter, 0.25-µm film thickness with DB-5 idle phase
Media phase	Helium with argon	Nitrogen
Injector	Autosampler Variant CP-8400	(manual)
Injection volume	5 µL	1 µL
Analysis methods	Internal standard	External standard

GC: Gas chromatography, ECD: Electron capture detector

Validation of analytical methods

Linearity tests

Linearity tests were performed with fenpropathrin at 20, 25, 30, 35, 40, and 45 ppb; lambda-cyhalothrin at 10, 15, 20, 25, 30, and 40 ppb; and chlorothalonil at 5, 10,15, 20, 25, and 30 ppb. Using plots of concentration versus area, the equations y=583.57x−4418.2 (r=0.9994), y=187.4x+12787 (r=0.9992), and y=1061.7x−177.8 (r=0.9993) were generated for the respective pesticides (Fig. 1).

LOD and LOQ

LOD and LOQ values for fenpropathrin, lambda-cyhalothrin, and chlorothalonil were 1.36 and 4.58, 1.45 and 4.85, and 1.12 and 3.73 ppb, respectively.

Accuracy and precision tests

The result of accuracy and precision test was summarized in Tables 2-7.

Establishment of sample concentrations

Extracted samples were analyzed using GC with an ECD. Analyses were performed in triplicate and no traces of pesticides were found in any of the samples (Table 8).

DISCUSSION

In the present study, we used GC-ECD instead of GC MS-MS because it has greater efficiency. Although GC-ECD is limited by the potential for the matrix to capture other electrons, the requirements of linearity were met in our analyses of fenpropathrin, lambda-cyhalothrin, and chlorothalonil, with r≥0.9990 in all cases [14].

LOD and LOQ of these analyses were 0.005 µg/kg and LOD 1.22 ppb, and these were better than in previous pesticide analyses of lettuces and oranges [14]. These LOD and LOQ values of the three pesticides indicate sufficient sensitivity of these analyses [14].

The present percentage RT and percentage VC values for the three pesticides were 70%–120% and ≤20%, respectively, and fulfilled the associated criteria. However, accuracy values did not increase in these analyses, likely reflecting technical limitations such as variations in additions of standards to vegetables, manual shaking and durations of waiting times, or variations in vortexing during extraction and partition stages.

No pesticide traces were found in any of the present samples, although chromatogram peaks with retention times of 10.78–10.79 min were

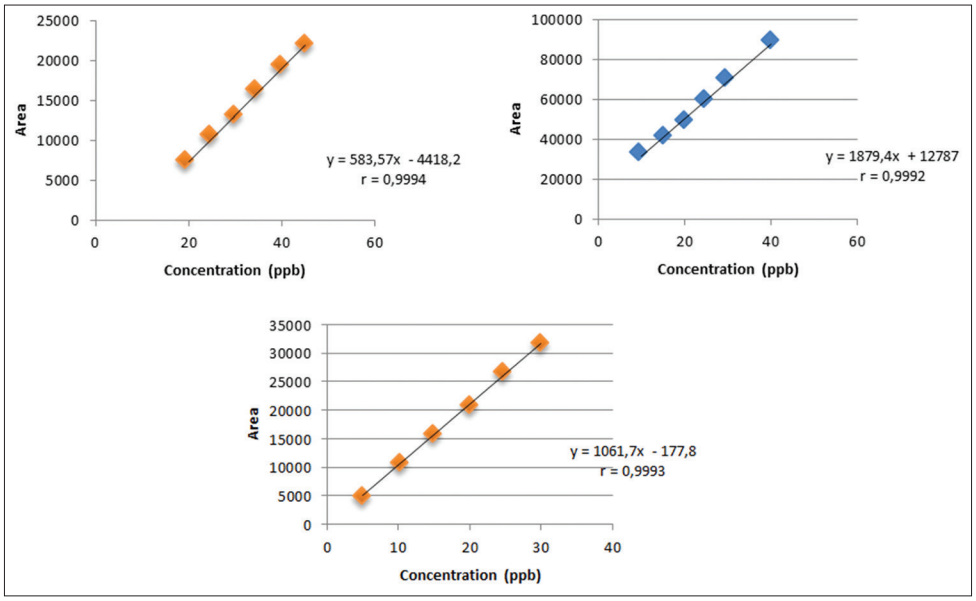

Fig. 1: Calibration curves of (a) fenpropathrin, (b) cyhalothrin, and (c) chlorothalonil

Table 2: Accuracy and precision tests for fenpropathrin in tomatoes

Standard Concentration (ppb)	Area	Standard Measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
20	8231.9	21.677	108.386	100.861	0.953	0.945
	6916.6	19.423	97.116			
	6708.1	19.066	95.330			
	7589.4	20.576	102.880			
	7102.1	19.741	98.705			
	7574.2	20.550	102.750			
35	12 754.8	29.427	84.079	86.819	3.295	3.795
	13 255.4	30.285	86.529			
	13 612.6	30.897	88.278			
	13 965.3	31.502	90.005			
	12 318.5	28.680	81.942			
	13 980.2	31.527	90.078			
45	15 891.5	34.803	77.339	79.124	4.035	5.099
	15 498.6	34.129	75.843			
	15 684.7	34.448	76.551			
	16 627.1	36.063	80.140			
	16087	35.138	78.083			
	18372.3	39.054	86.786			

Table 3: Accuracy and precision tests for fenpropathrin in potatoes

Standard concentration (ppb)	Area	Standard measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
20	6496.8	18.704	93.519	96.725	0.518	0.536
	6617	18.910	94.549			
	6849.1	19.308	96.538			
	6909	19.410	97.051			
	7354.9	20.174	100.871			
	6998.8	19.564	97.820			
35	13 735.3	31.108	88.879	85.540	3.325	3.888
	12 329.3	28.698	81.995			
	13 250.9	30.278	86.507			
	13 668.3	30.993	88.551			
	13 216.8	30.219	86.340			
	12 118.8	28.338	80.965			
45	17 440.2	37.456	83.236	85.779	0.882	1.028
	18 176.9	38.719	86.042			
	18 380.3	39.067	86.816			
	17 506.6	37.570	83.489			
	18 628.5	39.493	87.761			
	18 515.2	39.298	87.330			

Table 4: Accuracy and precision tests for Lambda-Cyhalothrin in Tomatoes

Standard Concentration (ppb)	Area	Standard Measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
10	29 410.4	8.845	88.451	93.095	1.305	1.401
	31 155.9	9.774	97.738			
	34 845.6	11.737	117.370			
	28 184.9	8.193	81.930			
	29 421.6	8.8510	88.510			
	28 680.7	8.458	84.568			
25	63 747.8	27.115	108.462	97.605	2.361	2.419
	50 397.5	20.012	80.048			
	59 506.5	24.859	99.435			
	60 362.7	25.314	101.257			
	59 458.4	24.833	99.333			
	58 407.4	24.274	97.096			
40	73 764.7	32.445	81.113	82.810	1.740	2.101
	74 468.1	32.820	82.049			
	77 827	34.607	86.517			
	71 340	31.155	77.888			
	72 799	31.931	79.829			
	80 044.3	35.787	89.467			

Table 5: Accuracy and precision tests for Lambda-Cyhalothrin in Potatoes

Standard Concentration (ppb)	Area	Standard Measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
10	34 865.4	11.748	117.476	114.526	0.383	0.335
	35 213.8	11.933	119.330			
	34 243.2	11.417	114.165			
	33 669.2	11.111	111.111			
	34 559.5	11.585	115.848			
	33 314.9	10.923	109.226			
25	47 224.1	18.323	73.294	86.710	3.247	3.745
	47 795.8	18.628	74.511			
	62 288.7	26.339	105.356			
	50 805.8	20.229	80.917			
	54 133.4	22.000	87.999			
	58 919	24.546	98.185			
40	63 613.4	27.044	90.147	100.448	3.599	3.583
	74 080	32.613	108.710			
	79 569.4	35.534	118.446			
	65 518.7	28.058	93.526			
	71 464.1	31.221	104.071			
	62 285.4	26.337	87.791			

Table 6: Accuracy and precision tests for Cyhalothrin in tomatoes

Standard Concentration (ppb)	Area	Standard Measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
5	4465.9	4.374	87.477	97.288	0.3604	0.370
	5300.4	5.160	103.197			
	4622.2	4.521	90.421			
	4954.4	4.834	96.679			
	5440.7	5.292	105.840			
	5136.7	5.006	100.113			
20	15 851.5	15.098	75.489	78.534	1.014	1.291
	16 439.5	15.651	78.258			
	15 134.9	14.423	72.114			
	18 136.8	17.250	86.251			
	17 319.6	16.481	82.403			
	16 106.8	15.338	76.691			
30	23 581.2	22.378	74.594	83.108	1.991	2.397
	25 828.6	24.495	81.650			
	27 123.5	25.715	85.716			
	26 812.5	25.422	84.739			
	29 671.3	28.114	93.715			
	24 740	23.470	78.232			

Table 7: Accuracy and precision tests for Cyhalothrin in potatoes

Standard Concentration (ppb)	Area	Standard measurement (ppb)	RT (%)	x̄	SD (%)	(VC) (%)
5	4439.2	4.349	86.974	84.884	0.354	0.417
	4366.3	4.280	85.600			
	3834.5	3.779	75.583			
	3938.6	3.877	77.546			
	4813.2	4.701	94.019			
	4577.7	4.479	89.583			
20	15 699.1	14.954	74.771	79.473	1.181	1.486
	16 132.8	15.363	76.814			
	18 476.2	17.570	87.850			
	15 191.5	14.476	72.381			
	17 718.8	16.857	84.283			
	16 966.3	16.148	80.740			
30	23 586.5	22.383	74.611	78.605	1.929	2.454
	23 213.1	22.032	73.439			
	24 066	22.835	76.116			
	28 829.8	27.322	91.073			
	24 407	23.157	77.187			
	25 050.3	23.762	79.206			

Table 8: Analyses of fenpropathrin, lambda-cyhalothrin, and chlorothalonil contents in tomatoes and potatoes

Commodities	Notes	Fenpropathrin	Lambda-Cyhalothrin	Chlorothalonil
Potatoes	Depok	Undetected	Undetected	Undetected
	Jakarta	Undetected	Undetected	Undetected
	Tangerang	Undetected	Undetected	Undetected
	Organic B	Undetected	Undetected	Undetected
	Organic H	Undetected	Undetected	Undetected
	Organic S	Undetected	Undetected	Undetected
Tomatoes	Depok	Undetected	Undetected	Undetected
	Jakarta	Undetected	Undetected	Undetected
	Tangerang	Undetected	Undetected	Undetected
	Organic O	Undetected	Undetected	Undetected
	Organic P	Undetected	Undetected	Undetected
	Organic S	Undetected	Undetected	Undetected

observed from several of the tomato and potato samples. This retention time range was similar to that of chlorothalonil (10.82–10.84) but was sufficiently different to conclude that the undefined peaks were not chromatogram peaks of chlorothalonil and were likely due to matrix components.

CONCLUSION

Our linearity tests showed that all standards met the requirement of r≥0.9990, and these were applicable to fenpropathrin, lambda-cyhalothrin, and chlorothalonil concentration ranges of 20–45 ppb,

10–40 ppb, and 5–30 ppb, respectively. LOD and LOQ values for fenpropathrin, lambda-cyhalothrin, and chlorothalonil were similar to those reported by Lozano *et al*. and indicated high sensitivity of analyses [12,13]. Accuracy and precision values also fulfilled the requirements, further validating of the methods used in this study. Finally, all samples fulfilled the requirements of SNI 7313: 2008 "Maximum Limit of Pesticides Residual Products in Harvest Commodities" and the other terms and conditions of Japanese standards.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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