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# Estimation of Pesticide Residues in Selected Products of Plant Origin from Poland with the Use of the HPLC-MS/MS Technique

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Received: 29 April 2020; Accepted: 27 May 2020; Published: 1 June 2020


**Abstract:** The purpose of this work was to compare the content of pesticide residues (250) in unprocessed plant products from farms situated in the eastern part of Poland. The content of pesticide residues in the analysed samples was assayed with the use of the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method combined with HPLC-MS/MS (high performance liquid chromatography with tandem mass spectrometry) analysis. The analyses revealed that among 160 analysed samples, pesticide residues were detected in 83 samples (approximately 52%), while in 77 samples (approximately 48%), no presence of those substances was noted. In all the samples in which the presence of the sought compounds was identified, their levels did not exceed the Maximum Residue Levels (MRL). The most often identified ones were azoxystrobin—detected in 36 samples (22.5%), linuron—assayed in 33 samples (20.6%), chlorpyrifos and carbendazim—each detected in 13 samples (8.1%), metalaxyl and metalaxyl M—in 11 samples (6.9%), and acetamiprid—in 7 samples (4.4%).

**Keywords:** pesticide residues; QuEChERS; LC-MS/MS; vegetables; fruits; herbs; spices

## 1. Introduction

The estimation of contaminants and chemical residues in food of plant origin assumes a significant importance, which is related with the progress of science and results from the critical attitude of consumers towards the applied methods of agricultural production and to the environmental pollution [1]. Accumulation of pesticide residues in food may cause toxic and allergic effects for human health and life as a result of the consumption of contaminated products [2]. For the protection of public health, the European Union introduced the highest permissible levels of pesticide residues in food and feed of plant and animal origin, regulated by the Regulation (EC) No. 396/2005 of the European Parliament and Council on 23 February 2005. Quantitative assays of pesticide residues in food allow the estimation of the exposure of consumers to the presence of those compounds and to perform risk assessment. The results of such analyses also provide important information on actual levels of pesticide residues and may cause a modification of the scope of their application in agriculture for the purpose of reduction of excessive levels relative to the Maximum Residue Levels (MRL). A highly important aspect in the estimation of the presence of pesticide residues is the application of a suitable analytical procedure that should meet the assumed requirements and guarantee the obtainment of results which can constitute the basis for making correct administrative decisions. Current studies in the field of estimation of pesticide residues indicate the universal character of the technique of liquid

chromatography with mass spectrometry (LC/MS/MS) in the analysis of that group of substances in samples of plant raw materials and in ready food products. Literature data confirm that the LC/MS/MS technique is characterised by adequate selectivity and specificity and allows to acquire, in the course of the analytical process, the required values of parameters confirming the quality of the result [3–10].

The quality requirements relating to food impose on the producers the necessity of controlling the quality of market products. Such a control results in an improvement of the quality of the food produced. One can also observe a trend towards minimisation of the number of plant protection treatments, but in spite of the existing legal regulations in this area, there are instances of breaking the regulations, resulting in the risk of products with exceeded limit levels for pesticide residues finding their way onto the market. In view of the above, the objective of this study was to compare the content of pesticide residues in 6 kinds of food products, i.e., vegetables, fruits, herbs, spices, and fruit and vegetable juices, as well as industrial plants originating from production farms in the eastern part of Poland.

## 2. Materials and Methods

### *2.1. Experimental Material*

The research material consisted of samples of unprocessed plant products collected at random from farms situated in the eastern part of Poland, in the period of 2015–2016. Imported spices and juices were purchased in Lublin supermarkets. Minimum weight of a sample was 3 kg. The total number of samples was 160, classified into 6 groups:

1. Vegetables (20)—carrot (2), cabbage (1), beetroot (2), root celery (1), parsley (2), green pea (1), cucumber (1), broccoli (1), pumpkin (3), beans (1), radish (1), chive (1), dill (1), peppers (1), field pea (1).
2. Fruits (26)—blackcurrant (9), cherry (2), strawberry (4), blueberry (1), aronia berry (1), apple (3), pear (2), raspberry (2), elderberry (2).
3. Herbs (85)—root of valerian (2), herbage of thyme (39), leaf of mint (3), root of common dandelion (4), leaf of lemon balm (3), herbage of common origanum (1), herbage of marjoram (1), fruit of coriander (3), linseed (17), leaf of small plantain (3), leaf of sage (1), herbage of rock rose (1), leaf of nettle (1) root of liquorice (1), flower of marigold (1), flower of elderberry (1), leaf of blackcurrant (2), leaf of purple coneflower (1).
4. Spices (22)—black pepper (4), bay leaf (1), orange skin (1), fruit of caraway (3), curcuma (1), nutmeg (1), allspice (1), ginger (1), herbal spice (4), herbal pepper substitute (3), Herbes de Provence (2).
5. Fruit and vegetable juices (4)—multifruit juice (1), pear juice (1), apple juice (1), beetroot juice (1).
6. Industrial plants (3)—wheat (2), rape (1).

### *2.2. Chemicals*

High-purity pesticide standards (250) were used for testing (98–99%, Dr. Ehrenstorfer GmbH, Augsburg, Niemcy; ChemService, West Chester, PA, USA): 2,4,5-T, 2,4-D, 2,4-DB, 3,5-Dichloroaniline, 3-hydroxycarbofuran, Abamectin, Acephate, Acetamiprid, Acrinathrin, Alachlor, Aldicarb, Aldicarb Sulfoxide, Aldicarb Sulphone, Ametryn, Amitraz, Atrazine, Azinophos-Ethyl, Azinophos-Methyl, Azoxystrobin, Benfuracarb, Bentazon, Benzoylprop ethyl, Bifenazate, Bromacil, Bromoxynil, Bromuconazole, Buprofezine, Butoxycarboxin, CAP (Captan), Carbaryl, Carbendazim, Carbetamide, Carbofuran, Carbosulfan, Carboxin, Chlorantraniliprole, Chloridazon, Chlorotoluron, Chlorpyrifos, Chlorsulfuron, Clofentezine, Clomazone, Clothianidin, Coumaphos, Cyanazine, Cyanofenphos, Cycloate, Cymoxanil, Cyphenothrin, Cyprofuram, DEF (Decafentin), Demeton-S-methyl, Demeton-S-methylsulphon, Desethyl atrazin, Desisopropyl atrazin, Desmedipham, Desmetryn, Diafenthiuron, Dialifos, Diazinon, Dicamba, Dichlofluanid, Dichloprop

(2.4-DP), Diclorvos, Dicrotophos, Diflubenzuron, Dimefuron, Dimethachlor, Dimethenamide, Dimethoate, Dimethomorph, Diniconazole, Diphenamide, Diphenylamine, Disulfoton, Ditalimfos, Diuron, DMF (2,4-Dimethyl-phenyl-formamidine), Dodine, Epoxiconazole, Etaconazole, Ethiofencarb, Ethirimol, Ethofenprox, Etoxazole, Etrimphos, Fenamidon, Fenamiphos, Fenazaquin, Fenbuconazole, Fenhexamid, Fenoxap-p-ethyl, Fenoxycarb, Fenpropimorph, Fenpyroximate, Fenthion, Fenthion sulfon, Fenuron, Fipronil, Flazasulfuron, Florosulam, Fluazifop, Fluazifop-p-butyl, Fluazinam, Fludioxonil, Flufenacet, Flufenoxuron, Fluometuron, Fluroxypyr, Flurtamon, Fluthiacet methyl, Flutriafol, Fonofos, Fosthiazate, Fuberidazol, Furathiocarb, Halfenprox, Haloxyfop, Haloxyfop methyl, Haloxyfop-2-ethoxyethyl, Heptenophos, Hexaflumuron, Hexazinone, Hexythiazox, Imazalil, Imazamox, Imazapyr, Imidacloprid, Indoxacarb, Ioxynil, Iprodione, Iprovalicarb, Isazofos, Isocarbamide, Isomethiozin, Isoproturon, Isoxaflutole, Lenacil, Linuron, Lufenuron, Malaoxon, Malathion, MCPA (2-Methyl-4-chlorophenoxyacetic acid), MCPB (4-(2-Methyl-4-chlorophenoxy) butyric acid), MCPP (Mecoprop), Mecarbam, Mepanipyrim, Metalaxyl, Metalaxyl-M, Metamitron, Metazachlor, Metconazol, Methabenzthiazuron, Methacrifos, Methamidophos, Methidathion, Methiocarb, Methoprotryne, Methoxyfenozide, Metobromuron, Metolachlor, Metolachlor S, Metosulam, Metoxuron, Metrafenon, Monocrotophos, Monolinuron, Monuron, Myclobutanil, Nicosulfuron, Nitenpyram, Norflurazon, Novaluron, Omethoate, Oxamyl, Oxycarboxin, Oxydemethon methyl, Paraoxon ethyl, Paraoxon methyl, Parathion ethyl, Pebulat, Penconazole, Pencycuron, Phenkapton, Phenmedipham, Phenothrin, Phenthoate, Phorate, Phosalone, Phosmet, Phosphamidon, Phoxim, Picoxystrobin, Pirimicarb, Pirimiphos methyl, Prochloraz, Profenofos, Prometryn, Propamocarb, Propanil, Propaquizafop, Prophos, Prosulfuron, Pyraclostrobin, Pyraflufen ethyl, Pyridaphenthion, Pyridate, Pyrimiphos ethyl, Pyriproxyfen, Quinmerac, Quizalofop-p-ethyl, Resmethrine, Rimsulfuron, Sebuthylazin, Sethoxydim, Siltiopham, Simazine, Simetryn, Spinosad A, Spinosad D, Spirotetramat, Spiroxamin, Sulfotep, Sulprofos, Tebuconazole, Tebufenozide, Tebufenpyrad, Tebutam, Teflubenzuron, Tepraloxydim, Terbucarb, Terbumeton, Terbuthialzine desethyl, Terbuthylazine, Tetramethrin, Thiabendazole, Thiacloprid, Thiamethoxam, Thiodicarb, Thiophanate methyl, Tolclofos methyl, Tolylfluanid, Triadimefon, Tri-allate, Triamiphos, Triazophos, Trichlorofon, Triclopyr, Trifloxystrobin, Triflumuron, Triforine. Standard solutions of pesticide in acetonitrile, with concentration of approximately 1000 mg $L^{-1}$, were prepared. Next, standard solutions of a mixture of pesticides in acetonitrile, with concentration of about 35 mg $L^{-1}$, were prepared for each of the compounds. Working standard solutions were prepared by diluting the standard mixtures of pesticide solutions with acetonitrile. All standard solutions were stored at temperatures lower than −20 °C. The choice of analysed pesticides resulted from the demand of herb producers' customers for analyses in line with the laboratory services market in the region. In addition, only pesticides for which the criteria for analytical quality were met were included in the analysis.

### *2.3. Preparation of Samples*

The analytical procedure was described in earlier work [11]. Portions of about 3 kg of plant material were suitably mixed to obtained uniform material, and then samples of approximately 100 g were collected and homogenised. The obtained homogenisate was transferred in suitable amounts to 50 mL test tubes. In the case of dry matrices, the samples were moistened to the level of about 95%.

The next step was the addition, to the homogenisate, of 10 mL of acetonitrile (Merck, Darmstadt, Germany) and 100 µL of internal standard of triphenylphosphate (Merck, Darmstadt, Germany) (10 µg $mL^{-1}$) assayed in the mode of positive ionisation and 100 µL of internal standard of bis-nitrophenyl urea (Merck) (10 µg $mL^{-1}$) assayed in the mode of negative ionisation as an internal standard. The test tube was shaken vigorously for 1 min. Next, a mixture of salts QuECheRS Mix I (Agilent Technologies, Santa Clara, CA, USA) was added, and the tube was shaken again for 1 min and centrifuged for 5 min (1361 rcf). The obtained extract was purified by adding the mixture of salts QuEChERS Mix II (Agilent Technologies, Santa Clara, CA, USA), while in the case of samples containing chlorophyll, the mixture QuEChERS Mix III (Agilent Technologies, Santa Clara, CA, USA)

was additionally added, and the tube was shaken again for 1 min, and then centrifuged for 5 min (1361 rcf). The extract prepared in this manner was transferred to the autosampler vial and subjected to chromatographic analysis.

### *2.4. Pesticides Analysis*

The content of pesticide residues in the analysed samples was assayed following a modified procedure developed in accordance with the standard PN-EN 15662:2008 [12], with the use of the method QuEChERS combined with LC-MS/MS analysis. The procedure applied in the study has been approved by the Polish Centre of Accreditation (PCA 1375).

HPLC MS/MS Analysis

A Shimadzu Prominence/20 series HPLC system (Shimadzu, Tokyo, Japan) and AB SCIEX 4000 QTRAP®LC-MS/MS system with Turbo V source (Foster City, California, USA) were used for LC-MS/MS analysis. The HPLC system was equipped with a LC-20 AD binary pump, a SIL-20 AC autosampler, a DGU-20A5 online degasser and a CTO-20A column oven. Nitrogen with a purity of at least 99% generated from a Peak Scientific nitro en generator (Billerica, MA, USA) was used in the ESI source and the collision cell. Analysis was performed using a 4.6 × 100 mm × 5 µm Agilent ZORBAX Eclipse XDB C18 column with a 10 µL injection. The column temperature was constant at 40 °C. A mobile phase gradient of water with 5 mM ammonium acetate and methanol with 5 mM ammonium formate and flow rate of 0.5 mL $min^{-1}$ were used. Mobile phase was composed of HPLC-grade water containing 5 mM ammonium acetate (eluent A) and HPLC-grade methanol containing 5 mM ammonium acetate (eluent B). The gradient elution was performed as follows: 0–0.1 min: 20% B; 0.1–1 min: 20–45% B; 1–9 min: 45–80% B; 9–19 min: 80–100% B, 19–20 min: 100% B; 20–21 min: 100–20% B; 21–24 min: 20% B. A flow rate of 0.5 mL $min^{-1}$ and an injection volume of 15 mL were used in the LC-MS/MS system.

The mass spectrometer was operated using an ESI source in the positive and negative mode. ESI parameters were as follows: ion spray voltage 5.5 kV (ESI+) and −4.5 kV (ESI−), source temperature 600 °C, curtain gas (nitrogen) 35 psi, ion source gas "1" 50 psi, ion source gas "2" 65 psi, and collision gas (nitrogen) 5 psi. ESI-MS/MS was operated in scheduled multiple reaction monitoring mode (MRM), in both positive and negative polarities, by scanning two precursor/products ion transitions for each target analyte. Both transitions were used for quantification and confirmation purposes (see the Supplementary Material: Tables S1 and S2).

The recovery for pesticides in the matrices tested ranged from 70% to 120%. The limit criterion for linearity was the range above $r \geq 0.995$ (values from 0.9950 to 0.9998 were obtained).

## 3. Results

The analyses revealed that among 160 analysed samples, pesticide residues were detected in 83 samples (approximately 52%), while in 77 samples (approximately 48%), no presence of those substances was noted. In all the samples in which the presence of the sought compounds was identified, their levels did not exceed the Maximum Residue Levels (MRL). The occurrence of the analysed contaminants in the particular kinds of analysed samples is presented in Table 1. Residues of plant protection agents were found most often in samples of fruits—approximately 70%, while in herbs and fruit juices, pesticides were noted in approximately 53% and 50% of the samples, respectively. The lowest share of samples containing that group of analysed contaminants was noted in the case of vegetables—40%, and spices—approximately 43% (Table 1). Among the food samples subjected to analysis, pesticide residues were most frequently detected: in the group of herbs—in thyme (80%), in the group of fruits—in blackcurrant (44.4%), and in the group of spices—in black pepper (44.4%) (Table 2). Residues of two or more pesticides were hound in 54 samples (65.1%). In total, the presence of two pesticides was found in 25 samples (30.12%), the presence of three pesticides was noted in 11 samples (13.3%), and the presence of four and five pesticides, in 8 and 6 samples, respectively

(9.6% and 7.2%). One each of the analysed samples contained combinations of 7, 8, 9, and 12 of the identified compounds (Table 2). Co-occurrence of pesticide residues was noted in 44 herbal samples (91.8%), in 4 fruit samples (14.8%), in 2 vegetable samples (10%), and in 2 spice samples (9.5%). In the case of the herbal samples, the most often detected combination was that of a fungicide and a herbicide (azoxystrobin and linuron)—28 samples (32.9%), a combination of 2 fungicides with a herbicide (azoxystrobin, carbendazim and linuron) was assayed in 8 samples (9.4%), and combinations of 2 fungicides with 2 herbicides (azoxystrobin, linuron, metalaxyl, and metalaxyl M) were found in 8 samples (9.4%).

The presence of residues of an insecticide (acetamiprid) and a fungicide (trifloxysrobin) was found in 4 samples of fruits, in 2 samples of vegetables, a combination of a fungicide (azoxystrobin) and a herbicide (linuron) was detected, and the occurrence of a fungicide (azoxystrobin) and a herbicide (linuron) was noted in 2 samples of spices. In individual samples of herbs, the most often detected pesticide residues were linuron and azoxysrobin, in fruit samples—thiacloprid and trifloxystrobin, in spice samples—metalaxyl, metalaxyl M, and chloropyrifos, while in vegetable samples—azoxystrobin and chlorpyrifos (Table 2).

In the analysed samples, a total of residues of 40 pesticides were identified. The most often identified ones were azoxystrobin—detected in 36 samples (22.5%), linuron—assayed in 33 samples (20.6%), chlorpyrifos and carbendazim—each detected in 13 samples (8.1%), metalaxyl and metalaxyl M—in 11 samples (6.9%), and acetamiprid—in 7 samples (4.4%). The frequency of occurrence of all identified pesticides is presented in Figure 1. From among the 250 compounds sought in the presented experiment, in the analysed samples, the presence of 40 pesticides was found, which means that no presence of 210 pesticides from the estimated group of plant protection agents was detected. In terms of the use of the marked substances, they were classified into groups: fungicides (47.5%), insecticides (32.5%), herbicides (15%), carbamates (2.5%), and organophosphorus pesticides (2.5%). In the presented research, all identified pesticide residues are authorised in Poland. All pesticides found in individual products of plant origin are dedicated to the protection of a given plant species.

**Table 1.** Number of samples with and without detected pesticides residues for each analysed food product.

| | **Food Product** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Vegetables** | | **Fruits** | | **Herbs** | | **Spices** | | **Fruit and Vegetable Juices** | | **Cereals** | | **Total** | |
| | **Number of Sumples** | **%** | **Number of Sumples** | **%** | **Number of Sumples** | **%** | **Number of Sumples** | **%** | **Number of Sumples** | **%** | **Number of Sumples** | **%** | **Number of Sumples** | **%** |
| Samples analysed | 20 | - | 27 | - | 85 | - | 21 | - | 4 | - | 3 | - | 160 | - |
| No residues found | 12 | 60 | 8 | 29.6 | 40 | 47.1 | 12 | 57.1 | 2 | 50 | 3 | 100 | 77 | 48.1 |
| Residues found < MRL | 8 | 40 | 19 | 70.4 | 45 | 52.9 | 9 | 42.9 | 2 | 50 | 0 | 0 | 83 | 51.9 |
| Residues found > MRL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

MRL—Maximum Residue Levels.

**Table 2.** Pesticide residues concentration in examined food samples.

| No. | Food Product | Pesticide Residue | MRL (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Concentration (mg kg$^{-1}$) | Uncertainty (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| | | | **Herbs** | | | |
| 1 | Thyme herb | Acetamiprid | 3.0 | 0.0001 | 0.026 | ±0.005 |
| | | Azoxystrobin | 70.0 | 0.0001 | 0.073 | ±0.026 |
| | | Carbendazim | 0.1 | 0.0001 | 0.052 | ±0.016 |
| | | Chlorpyriphos | 0.05 | 0.0001 | 0.012 | ±0.003 |
| | | Dimethoate | 0.02 | 0.0001 | 0.010 | ±0.003 |
| | | Linuron | 1.0 | 0.0002 | 0.014 | ±0.005 |
| | | Metalaxyl | 2.0 | 0.0001 | 0.046 | ±0.009 |
| 2 | Thyme herb | Azoxystrobin | 70 | 0.005 | 0.023 | ±0.008 |
| 3 | Thyme herb | Acetamiprid | 3.0 | 0.0001 | 0.018 | ±0.004 |
| | | Azoxystrobin | 70.0 | 0.0001 | 0.052 | ±0.018 |
| | | Carbendazim | 0.1 | 0.0001 | 0.027 | ±0.008 |
| | | Linuron | 1.0 | 0.0002 | 0.015 | ±0.005 |
| 4 | Thyme herb | Azoxystrobin | 70 | 0.0001 | 0.035 | ±0.012 |
| | | Carbendazim | 0.1 | 0.0001 | 0.042 | ±0.013 |
| | | Linuron | 1.0 | 0.0002 | 0.009 | ±0.012 |
| | | Metalaxyl | 2.0 | 0.0001 | 0.013 | ±0.003 |
| | | Metazachlor | 0.3 | 0.0001 | 0.024 | ±0.006 |
| 5 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.069 | ±0.024 |
| | | Linuron | 1.0 | 0.005 | 0.026 | ±0.008 |
| 6 | Thyme herb | Linuron | 1.0 | 0.005 | 0.057 | ±0.018 |
| 7 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.036 | ±0.017 |
| | | Linuron | 1.0 | 0.005 | 0.031 | ±0.012 |
| 8 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.098 | ±0.034 |
| | | Linuron | 1.0 | 0.005 | 0.022 | ±0.009 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.028 | ±0.006 |
| | | Metalaksyl M | | 0.002 | 0.027 | ±0.005 |
| 9 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.013 | ±0.005 |
| | | Carbendazim | 0.1 | 0.002 | 0.08 | ±0.034 |
| | | Linuron | 1.0 | 0.005 | 0.032 | ±0.012 |
| 10 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.028 | ±0.010 |
| | | Carbendazim | 0.1 | 0.002 | 0.093 | ±0.035 |
| | | Linuron | 1.0 | 0.005 | 0.019 | ±0.007 |
| 11 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.042 | ±0.02 |
| | | Carbendazim | 0.1 | 0.002 | 0.022 | ±0.007 |
| | | Linuron | 1.0 | 0.005 | 0.027 | ±0.01 |
| | | Pyraclostrobin | 2.0 | 0.002 | 0.022 | ±0.007 |
| 12 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.009 | ±0.002 |
| | | Chlorantraniliprole | 20.0 | 0.005 | 0.270 | ±0.130 |
| | | Dimethoate | 0.02 | 0.002 | 0.140 | ±0.040 |
| | | Linuron | 1.0 | 0.005 | 0.012 | ±0.004 |
| 13 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.007 | ±0.001 |
| | | Linuron | 1.0 | 0.005 | 0.03 | ±0.010 |
| 14 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.053 | ±0.018 |
| | | Carbendazim | 0.1 | 0.002 | 0.021 | ±0.007 |
| 15 | Thyme herb | Metalaxyl | 2.0 * | 0.002 | 0.073 | ±0.015 |
| | | Metalaxyl-M | | 0.002 | 0.073 | ±0.015 |
| 16 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.031 | ±0.015 |
| | | Carbendazim | 0.1 | 0.002 | 0.086 | ±0.033 |
| | | Linuron | 1.0 | 0.005 | 0.029 | ±0.009 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.018 | ±0.004 |
| | | Metalaxyl-M | | 0.002 | 0.018 | ±0.004 |

**Table 2.** *Cont.*

| No. | Food Product | Pesticide Residue | MRL (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Concentration (mg kg$^{-1}$) | Uncertainty (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 17 | Thyme herb | Chlorantraniliprole | 20.0 | 0.005 | 0.17 | ±0.080 |
| | | Linuron | 1.0 | 0.005 | 0.061 | ±0.019 |
| 18 | Thyme herb | Carbendazim | 0.1 | 0.002 | 0.062 | ±0.019 |
| | | Chlorantraniliprole | 20.0 | 0.005 | 0.160 | ±0.07 |
| 19 | Thyme herb | Carbendazim | 0.1 | 0.002 | 1.29 | ±0.005 |
| | | Linuron | 1.0 | 0.005 | 0.016 | ±0.005 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.008 | ±0.002 |
| | | Metalaxyl-M | | 0.002 | 0.006 | ±0.002 |
| 20 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.067 | ±0.013 |
| | | Linuron | 1.0 | 0.005 | 0.110 | ±0.030 |
| 21 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.035 | ±0.007 |
| | | Linuron | 1.0 | 0.005 | 0.120 | ±0.040 |
| 22 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.013 | ±0.003 |
| | | Linuron | 1.0 | 0.005 | 0.021 | ±0.006 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.058 | ±0.017 |
| | | Metalaxyl-M | | 0.002 | 0.060 | ±0.019 |
| 23 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.041 | ±0.008 |
| | | Carbendazim | 0.1 | 0.002 | 0.022 | ±0.007 |
| | | Chlorantraniliprole | 20.0 | 0.005 | 0.094 | ±0.043 |
| | | Chlorotoluron | 0.02 | 0.002 | 0.009 | ±0.002 |
| | | Linuron | 1.0 | 0.005 | 0.062 | ±0.019 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.015 | ±0.004 |
| | | Metalaxyl-M | | 0.002 | 0.013 | ±0.004 |
| | | Metolachlor | 0.05 * | 0.005 | 0.012 | ±0.003 |
| | | Metolachlor S | | 0.002 | <LOQ = 0.002 | ±0.002 |
| 24 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.020 | ±0.004 |
| | | Linuron | 1.0 | 0.005 | 0.016 | ±0.005 |
| 25 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.050 | ±0.01 |
| | | Linuron | 1.0 | 0.005 | 0.100 | ±0.003 |
| 26 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.210 | ±0.040 |
| | | Linuron | 1.0 | 0.005 | 0.026 | ±0.008 |
| 27 | Thyme herb | Linuron | 1.0 | 0.005 | 0.110 | ±0.030 |
| 28 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.009 | ±0.002 |
| | | Linuron | 1.0 | 0.005 | 0.014 | ±0.004 |
| 29 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.290 | ±0.060 |
| | | Linuron | 1.0 | 0.005 | 0.015 | ±0.005 |
| 30 | Thyme herb | Linuron | 1.0 | 0.005 | 0.085 | ±0.026 |
| 31 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.059 | ±0.012 |
| | | Linuron | 1.0 | 0.005 | 0.008 | ±0.002 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.018 | ±0.005 |
| | | Metalaxyl-M | | 0.002 | 0.015 | ±0.005 |
| 32 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.330 | ±0.110 |
| | | Linuron | 1.0 | 0.005 | 0.015 | ±0.005 |
| 33 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.044 | ±0.009 |
| | | Linuron | 1.0 | 0.005 | 0.013 | ±0.004 |
| 34 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.230 | ±0.080 |
| | | Linuron | 1.0 | 0.005 | 0.018 | ±0.006 |
| 35 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.290 | ±0.099 |
| | | Linuron | 1.0 | 0.005 | 0.048 | ±0.015 |
| | | Metalaxyl | 2.0 * | 0.002 | 0.11 | ±0.030 |
| | | Metalaxyl-M | | 0.002 | 0.12 | ±0.040 |
| | | Trifloxystrobin | 15.0 | 0.002 | 0.015 | ±0.003 |

**Table 2.** *Cont.*

| No. | Food Product | Pesticide Residue | MRL (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Concentration (mg kg$^{-1}$) | Uncertainty (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 36 | Thyme herb | Azoxystrobin | 70.0 | 0.005 | 0.27 | ±0.092 |
| | | Linuron | 1.0 | 0.005 | 0.029 | ±0.009 |
| 37 | Blackcurrant leaf | Azoxystrobin | 5.0 | 0.005 | 1.530 | ±0.520 |
| | | Linuron | 0.05 | 0.005 | 0.160 | ±0.050 |
| | | Tebuconazole | 1.5 | 0.005 | 0.015 | ±0.004 |
| 38 | Blackcurrant leaf | Azoxystrobin | 5.0 | 0.005 | 1.620 | ±0.550 |
| | | Clomazone | 0.01 | 0.005 | 0.038 | ±0.010 |
| | | Linuron | 0.05 | 0.005 | 0.290 | ±0.090 |
| | | Tebuconazole | 1.5 | 0.005 | 0.051 | ±0.013 |
| 39 | Valerian root | Azoxystrobin | 50.0 | 0.005 | 0.210 | ±0.070 |
| 40 | Coriander fruit | Azoxystrobin | 70.0 | 0.005 | 0.009 | ±0.002 |
| 41 | Elderbery flower | Picoxystrobin | 0.01 | 0.005 | 0.009 | ±0.002 |
| 42 | Purple coneflower leaf | Chlorpyrifos | 0.05 | 0.002 | 0.043 | ±0.010 |
| 43 | Sage leaf | Linuron | 1.0 | 0.005 | 0.012 | ±0.004 |
| 44 | Linseed | Epoxiconazole | 0.05 | 0.005 | 0.010 | ±0.003 |
| 45 | Linseed | Chlorpyrifos | 0.05 | 0.005 | 0.050 | ±0.012 |
| | | | Fruits | | | |
| 46 | Blackcurrant | Thiacloprid | 1.0 | 0.002 | 0.060 | ±0.022 |
| 47 | Blackcurrant | Thiacloprid | 1.0 | 0.002 | 0.050 | ±0.019 |
| 48 | Blackcurrant | Fenpyroximate | 1.0 | 0.002 | 0.027 | ±0.009 |
| | | Thiacloprid | 1.0 | 0.002 | 0.022 | ±0.004 |
| | | Trifloxystrobin | 1.0 | 0.002 | 0.021 | ±0.006 |
| 49 | Blackcurrant | Thiacloprid | 1.0 | 0.002 | 0.016 | ±0.006 |
| 50 | Blackcurrant | Acetamiprid | 2.0 | 0.002 | 0.016 | ±0.003 |
| | | Trifloxystrobin | 1.0 | 0.002 | 0.070 | ±0.030 |
| 51 | Blackcurrant | Acetamiprid | 2.0 | 0.002 | 0.023 | ±0.005 |
| 52 | Blackcurrant | Acetamiprid | 2.0 | 0.002 | 0.011 | ±0.002 |
| | | Thiacloprid | 1.0 | 0.002 | 0.066 | ±0.024 |
| 53 | Blackcurrant | Fenpyroximate | 1.0 | 0.002 | 0.040 | ±0.013 |
| 54 | Cherry | Dodine | 5.0 | 0.002 | 0.087 | ±0.018 |
| | | Thiacloprid | 0.02 | 0.002 | 0.003 | ±0.001 |
| 55 | Cherry | Dodine | 5.0 | 0.002 | 0.037 | ±0.008 |
| 56 | Strawberry | Acetamiprid | 0.5 | 0.001 | 0.005 | ±0.001 |
| | | Azoxystrobin | 10.0 | 0.0001 | 0.100 | ±0.034 |
| | | Chlorotoluron | 0.01 | 0.0005 | 0.009 | ±0.002 |
| | | Cyprodinil | 5.0 | 0.001 | 0.150 | ±0.038 |
| | | Difenoconazole | 0.4 | 0.0002 | 0.063 | ±0.016 |
| | | Fludioxonil | 4.0 | 0.0001 | 0.200 | ±0.068 |
| | | Mepanipyrim | 1.5 | 0.0001 | 0.080 | ±0.026 |
| | | Trifloxystrobin | 1.0 | 0.0001 | 0.330 | ±0.092 |
| 57 | Apple | Diflubenzuron | 0.05 | 0.01 | 0.042 | ±0.011 |
| | | Fenpyroximate | 0.05 | 0.002 | 0.013 | ±0.004 |
| | | Fenpropimorph | 0.05 | 0.002 | 0.011 | ±0.003 |
| | | Teflubenzuron | 2.0 | 0.01 | 0.046 | ±0.012 |
| | | Triflumuron | 2.0 | 0.002 | 0.063 | ±0.016 |

**Table 2.** *Cont.*

| No. | Food Product | Pesticide Residue | MRL (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Concentration (mg kg$^{-1}$) | Uncertainty (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 58 | Apple | Acetamiprid | 0.8 | 0.002 | 0.024 | ±0.005 |
| | | Carbendazim | 0.2 | 0.002 | 0.098 | ±0.030 |
| | | Chlorpyrifos | 0.5 | 0.002 | 0.085 | ±0.020 |
| | | Diflubenzuron | 5.0 | 0.01 | 0.014 | ±0.004 |
| | | Fenpyroximate | 0.3 | 0.002 | 0.018 | ±0.006 |
| | | Fludioxonil | 5.0 | 0.002 | 0.013 | ±0.004 |
| | | Methoxyfenozide | 2.0 | 0.002 | 0.064 | ±0.016 |
| | | Pirimicarb | 2.0 | 0.002 | 0.020 | ±0.005 |
| | | Pyraclostrobin | 0.5 | 0.002 | 0.039 | ±0.012 |
| | | Thiacloprid | 0.3 | 0.005 | 0.023 | ±0.009 |
| | | Tebuconazole | 0.3 | 0.002 | 0.025 | ±0.006 |
| | | Trifloxystrobin | 0.5 | | 0.025 | ±0.007 |
| 59 | Apple | Carbendazim | 0.2 | 0.002 | 0.062 | ±0.019 |
| 60 | Strawberry | Azoxystrobin | 60.0 | 0.005 | 0.009 | ±0.003 |
| 61 | Strawberry | Fludioxonil | 4.0 | 0.002 | 0.043 | ±0.015 |
| 62 | Raspberry | Imidacloprid | 5.0 | 0.005 | 0.009 | ±0.003 |
| | | Thiamethoxam | 0.05 | 0.002 | 0.009 | ±0.003 |
| 63 | Elderberry | Chlorpyrifos | 0.05 | 0.002 | 0.015 | ±0.003 |
| | | | Spices | | | |
| 64 | Black pepper | Metalaxyl | 0.1 * | 0.002 | 0.016 | ±0.003 |
| | | Metalaxyl-M | | 0.002 | 0.015 | ±0.003 |
| 65 | Black pepper | Acetamiprid | 0.05 | 0.002 | 0.012 | ±0.002 |
| | | Azoxystrobin | 0.3 | 0.005 | 0.022 | ±0.007 |
| | | Carbofuran | 0.05 | 0.002 | 0.015 | ±0.004 |
| | | Metalaxyl | 0.1 * | 0.002 | 0.019 | ±0.004 |
| | | Metalaxyl-M | | 0.002 | 0.018 | ±0.004 |
| 66 | Black pepper | Metalaxyl | 0.1 * | 0.002 | 0.041 | ±0.008 |
| | | Metalaxyl-M | | 0.002 | 0.039 | ±0.008 |
| 67 | Black pepper | Metalaxyl | 0.1 * | 0.002 | 0.011 | ±0.002 |
| | | Metalaxyl-M | | 0.002 | 0.010 | ±0.002 |
| 68 | Orange skin | Imazalil | 5.0 | 0.002 | 3.090 | ±0.772 |
| | | Prochloraz | 10.0 | 0.002 | 0.071 | ±0.018 |
| | | Thiabendazole | 5.0 | 0.005 | 2.020 | ±0.505 |
| 70 | Curcuma | Chlorpyrifos | 1.0 | 0.002 | 0.042 | ±0.010 |
| 69 | Caraway fruit | Chlorpyrifos | 1.0 | 0.002 | 0.051 | ±0.012 |
| 71 | Caraway fruit | Acetamiprid | 0.05 | 0.002 | 0.99 | ±0.200 |
| | | Azoxystrobin | 0.3 | 0.005 | 0.014 | ±0.003 |
| | | Carbendazim | 0.1 | 0.002 | 1.50 | ±0.450 |
| | | Chlorpyrifos | 1.0 | 0.002 | 0.095 | ±0.022 |
| | | Thiamethoxam | 0.05 | 0.002 | 0.100 | ±0.030 |
| 72 | Caraway fruit | Carbendazim | 0.1 | 0.002 | 0.048 | ±0.014 |
| | | Chlorpyrifos | 1.0 | 0.002 | 0.028 | ±0.006 |
| | | Fenpropimorph | 0.1 | 0.002 | 0.011 | ±0.003 |
| | | | **Vegetables** | | | |
| 73 | Carrot | Chlorpyrifos | 0.1 | 0.002 | 0.013 | ±0.006 |
| 74 | Beetroot | Tebuconazole | 0.02 | 0.005 | 0.008 | ±0.002 |
| 75 | Celery root | Azoxystrobin | 1.0 | 0.005 | 0.007 | ±0.003 |
| | | Linuron | 0.5 | 0.005 | 0.027 | ±0.010 |
| | | Tebuconazole | 0.5 | 0.005 | 0.013 | ±0.004 |
| 76 | Parsley root | Linuron | 0.2 | 0.005 | 0.038 | ±0.013 |
| 77 | Broccoli | Chlorpyrifos | 0.05 | 0.002 | 0.200 | ±0.048 |

**Table 2.** *Cont.*

| No. | Food Product | Pesticide Residue | MRL (mg kg$^{-1}$) | LOQ (mg kg$^{-1}$) | Concentration (mg kg$^{-1}$) | Uncertainty (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 78 | Radish | Metalaxyl | 0.1 * | 0.002 | 0.011 | ±0.004 |
| | | Metalaxyl-M | | 0.002 | 0.011 | ±0.003 |
| | | Pyraclostrobin | 0.5 | 0.002 | 0.016 | ±0.005 |
| 79 | Chive | Azoxystrobin | 70.0 | 0.005 | 0.051 | ±0.024 |
| | | Imidacloprid | 2.0 | 0.005 | 0.009 | ±0.003 |
| | | Linuron | 1.0 | 0.005 | 0.007 | ±0.002 |
| 80 | Dill | Azoxystrobin | 0.3 | 0.005 | 0.028 | ±0.013 |
| | | Chlorpyrifos | 5.0 | 0.002 | 0.019 | ±0.006 |
| | | Mepanipyrim | 0.05 | 0.002 | 0.012 | ±0.004 |
| 81 | Parsley root | Azoxystrobin | 70.0 | 0.005 | 0.050 | ±0.024 |
| | | Chlorpyrifos | 0.05 | 0.002 | 0.220 | ±0.090 |
| **Fruit and vegetable juices** | | | | | | |
| 82 | Pear juice | Acetamiprid | 0.8 | 0.0001 | 0.010 | ±0.004 |
| | | Bosacalid | 2.0 | 0.0005 | 0.015 | ±0.004 |
| | | Clothianidin | 0.4 | 0.0005 | 0.008 | ±0.003 |
| 83 | Beetroot juice | Tebuconazole | 0.02 | 0.0001 | 0.068 | ±0.017 |

LOQ—The Limit of Quantification, MRL—Maximum Residue Levels, * sum of Metalaxyl and Metalaxyl-M.

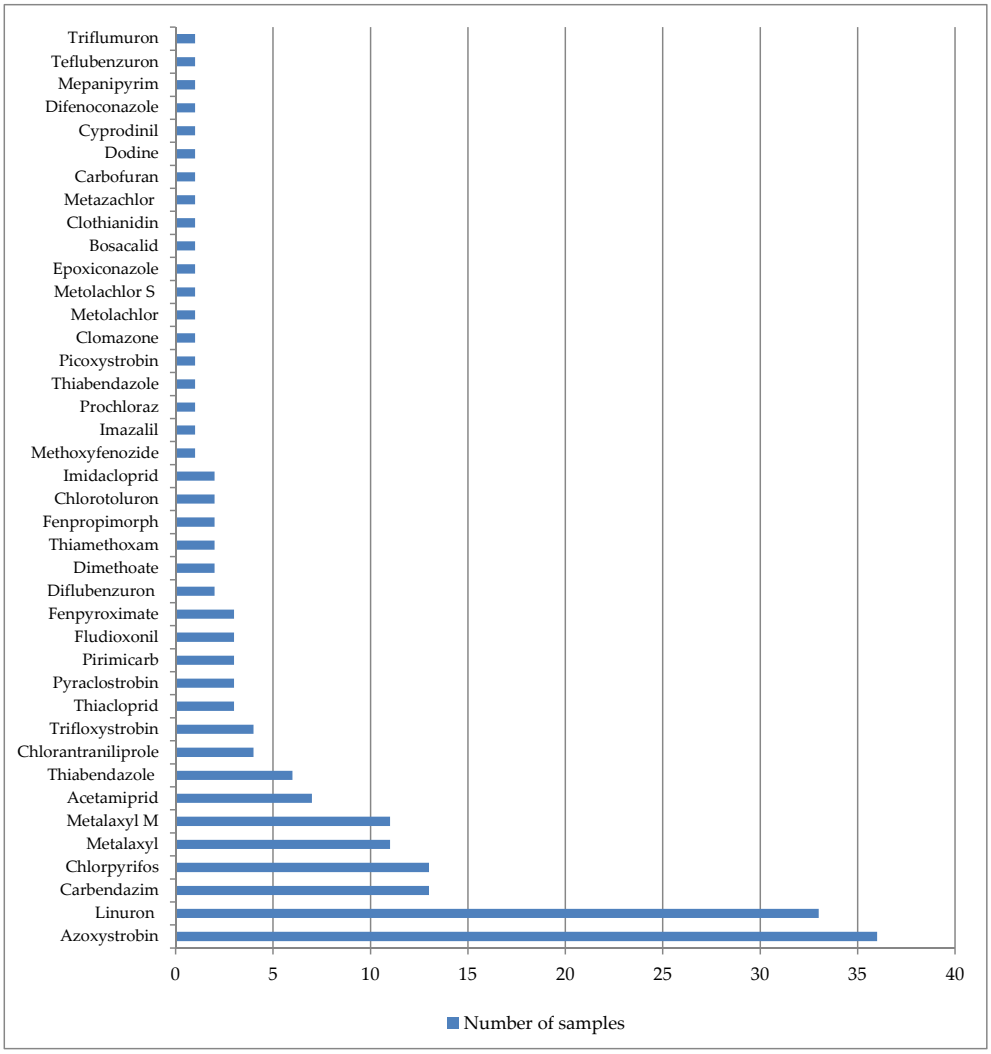


**Figure 1.** Pesticide occurrence frequency in analysed samples.

## 4. Discussion

In the presented study, the percentage share of samples containing pesticide residues (42.9–66.7%) correlates with the results obtained by other authors for the criterion "kind of sample"—Table 3. In studies concerned with vegetables, the percentage share of samples in which pesticide residues were noted varied from 15.9% to 77.8% (Table 1). Similar results were obtained in studies including fruit samples, for which the presence of pesticide residues was from 33.3 % to 77.4% of cases (Table 3). Referring to earlier results from studies covering samples of fruits and vegetables, it was demonstrated that pesticide residues in vegetables were less frequently found than in fruits [13,14], which is also supported by the results obtained in the presented experiment. Similar data were published by the European Food Safety Authority (EFSA) in 2014 and 2015, in the area of control studies on pesticide residues in food products in the member states of the European Union, indicating the presence of pesticide residues in 49–53% of samples of vegetables. Comparative studies on conventional and organic cultivations also confirmed a higher frequency of occurrence of pesticide residues in samples of fruits (75% and 25.8%) in relation to samples of vegetables (32% and 8.7%) [15,16]. The cause for this is attributed by those authors to the probability of application of a higher concentration of plant protection agents with extended effect duration, as well as to the use of various spraying technologies which may contribute to an increased accumulation of pesticide residues in fruits. A compilation of numerical data concerning the observed presence of various pesticide residues in food samples is presented in Figure 2. In the study, the own group of pesticides was most often determined as fungicides—47.5%, while every third designated plant protection product was an insecticide (32.5%). Fungicides dominated in samples from domestic primary production, tested by Dyjak et al. [17] in 2017 and Nowacka et al. [18] in 2011, as they constituted 45.5% and 63.9% respectively, and insecticides—24.5% and 32.5%. Also, in studies conducted by Szpyrk et al. [19], fungicides occurred as the most common pesticide residues. Analysing the frequency of occurrence of various pesticide residues in samples of fruits and vegetables (Figure 2), the most frequently identified pesticides were: chlorpyrifos (25%), cypermethrin (16.7%), imazalil (16.7%), azoxystrobin (12.5%), carbendazim (12.5%), imidacloprid (8.3%), cyprodinil (8.3%), permethrin (8.3%) and pyridaben (8.3%), enosulfan (4.2%), difenoconazole (4.2%), haloxyfop-R-Methyl (4.2%), boscalid (4.2%), chlorothalonil (4.2%), phosalone (4.2%), ∑-HCH (4.2%), diazinon (4.2%), enthoprophos (4.2%), pendimethalin (4.2%), acequinocyl (4.2%), iprodione (4.2%), bifenthrin (4.2%), deltamethrin (4.2%), metalaxyl (4.2%), and thiabendazole (4.2%). Four of those—azoxystrobin, carbendazim, chlorpyrifos, and metalaxyl—were also among the most frequently identified pesticides in the presented study (Figure 1). Authors conducting research on the presence of pesticide residues in plant samples also confirm the presence of those pesticides in samples of fruits and vegetables, in food of plant origin, in diet supplements, and also in plant samples used in Chinese medicine (Table 3). In the group of analysed fruits, pesticide residues were most frequently identified in samples of blackcurrant (44.4%), which is also reported in a study conducted in Poland in the years 2010–2015, in which the highest percentage level of pesticide residues among all of the analysed samples was demonstrated in blackcurrant—50% [15,20], and in black and red currant—40.9% [14]. In the presented study, the level of detected pesticide residues in herbs (52.9%) and spices (42.7%) correlates with the results obtained in study by Reinholds et al. [21] and Kowalska [11], who demonstrated the presence of pesticides in 59% and 71% of analysed samples of herbs and spices. The number of detected pesticide residues in herbs varied from 1 to 7 compounds in an individual sample of thyme (Table 2). Only in 3 (7.7%) among the 39 analysed samples of thyme was no presence found of the plant protection agents from the group analysed in their experiment. The remaining 36 samples contained pesticides, which is also confirmed by the study of Reinholds et al. [21], which showed similar values of pesticide residues in the analysed samples of that raw material (82%). In our study, the most frequently identified pesticides were linuron and azoxystrobin, while in studies by other authors, presence of plant procection, such as cymoxanil, dimethoate, and tebuconazole, was found [21–23]. Studies conducted in Poland have demonstrated the presence of the same pesticides in the analysed herb samples—azoxystrobin and linuron [11].

In the group of analysed spices, the sought compounds were detected most frequently in samples of black pepper (44.4%). In 4 out 7 analysed samples, the presence of pesticide residues was found, which is supported by the study by Ferrer-Amate et al. [24], and Reinholds et al. [21], who obtained similar results for samples of that spice.

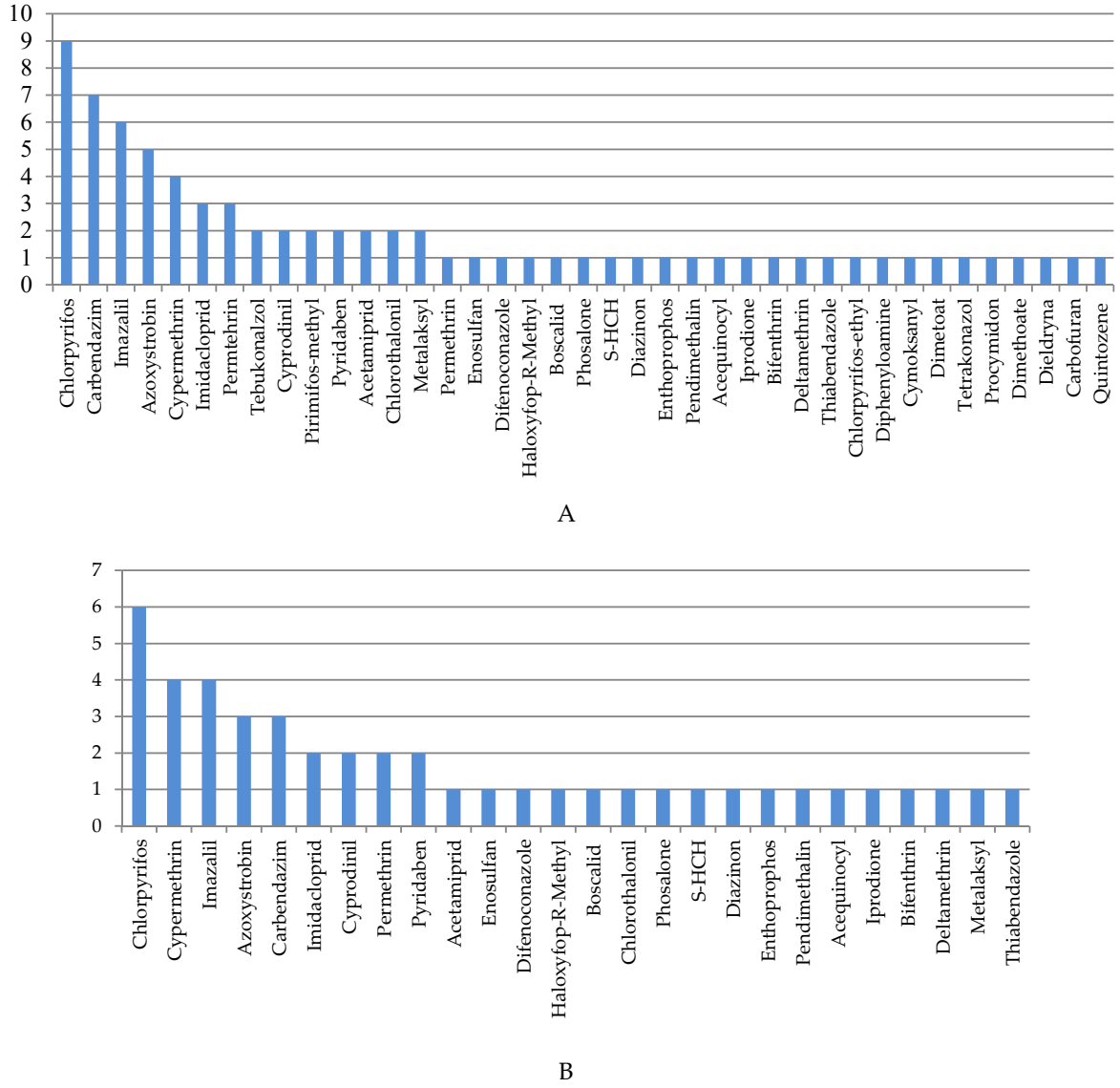


**Figure 2.** The most frequently detected pesticide residues in samples of plant origin according to literature data, (**A**) in all samples, (**B**) in fruit and vegetable samples (literature reports in Table 3).

**Table 3.** Summary of the most frequently detected pesticides in different food samples reported in the literature.

| No. | Food Category | No. of Samples | No. of Samples with Detected Residues | No. of Analysed Pesticides | No. of Detected Pesticides | Most Frequently Found Pesticide | % (1) | % (2) | References |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Vegetables | 1057 | 168 | 86 | 43 | Permethrin<br>Enosulfan | 15.9 | 50.0 | [25] |
| 2 | Vegetables | 30 | 5 | 283 | 4 | Cypermethrin<br>Chlorpyrifos<br>Difenoconazole | 16.7 | 1.4 | [26] |
| 3 | Vegetables (bean) | 178 | 39 | 58 | 39 | Cyprodinil, Haloxyfop-R-Methyl | 21.9 | 67.2 | [27] |
| 4 | Vegetables | 365 | 118 | 130 | 15 | Chlorpyrifos<br>Cypermethrin | 32.3 | 11.5 | [28] |
| 5 | Vegetables | 138 | 47 | 242 | 17 | Azoxystrobin Boscalid Chlorothalonil | 34.1 | 7.0 | [29] |
| 6 | Vegetables (tomato) | 20 | 8 | 30 | 6 | Azoxystrobin<br>Cyprodinil | 40.0 | 20.0 | [8] |
| 7 | Vegetables | 90 | 70 | 18 | 14 | Chlorpyrifos<br>Phosalone | 77.8 | 77.8 | [10] |
| 8 | Vegetables | 20 | Not defined | 48 | 23 | Σ-HCH, Permethrin | - | 47.9 | [30] |
| 9 | Fruit (peach) | 1150 | 383 | 31 | 22 | Chlorpyrifos<br>Diazinon | 33.3 | 71.0 | [31] |
| 10 | Omija fruit and juice | 420 | 143 | 33 | 4 | Enthoprophos<br>Pendimethalin | 34.1 | 12.1 | [32] |
| 11 | Yuza fruits and tea | 155 | 120 | 7 | 3 | Carbendazim<br>Acequinocyl | 77.4 | 42.9 | [7] |
| 12 | Fruits and vegetables | 199 | 46 | 74 | Not defined | Imazalil<br>Iprodione<br>Azoxystrobin | 23.1 | - | [33] |
| 13 | Fruits and vegetables | 20 | 5 | 82 | 36 | Pyridaben | 25.0 | 43.9 | [34] |
| 14 | Fruits and vegetables | 144 | 46 | 60 | 15 | Carbendazim<br>Acetamiprid | 31.9 | 25.0 | [20] |
| 15 | Fruits and vegetables | 866 | 293 | 102 | 30 | Imazalil | 33.8 | 29.4 | [35] |

**Table 3.** *Cont.*

| No. | Food Category | No. of Samples | No. of Samples with Detected Residues | No. of Analysed Pesticides | No. of Detected Pesticides | Most Frequently Found Pesticide | % (1) | % (2) | References |
|---|---|---|---|---|---|---|---|---|---|
| 16 | Fruits and vegetables | 3009 | 1135 | 22 | 22 | Cypermethrin | 37.7 | 100.0 | [36] |
| 17 | Fruits and vegetables | 1463 | 689 | 121 | 44 | Bifenthrin<br>Pyridaben | 47.1 | 36.4 | [37] |
| 18 | Fruits and vegetables | 13,556 | 6548 | 229 | 15 | Carbendazim<br>Chlorpyrifos | 48.3 | 6.6 | [6] |
| 19 | Fruits and vegetables | 150 | 88 | 34 | 16 | Deltamethrin<br>Imidacloprid<br>Cypermethrin<br>Chlorpyrifos<br>Metalaksyl | 58.7 | 47.1 | [38] |
| 20 | Fruits and vegetables | 724 | 586 | 326 | 83 | Thiabendazole<br>Imazalil | 80.9 | 25.5 | [5] |
| 21 | Fruits and vegetables | 17 | 17 | 100 | 26 | Imazalil<br>Imidacloprid | 100.0 | 26.0 | [9] |
| 22 | Herbs | 30 | 2/3 | 155 | 3 | Chlorpyrifos-ethyl<br>Diphenyloamine<br>Tebukonazol | 6.7–10.0 | 1.9 | [22] |
| 23 | Herbs and spices | 300 | 177 | 134 | 24 | Cymoksanyl<br>Dimetoat<br>Tebukonazol<br>Tetrakonazol | 59.0 | 17.9 | [21] |
| 24 | Herbs | 104 | 75 | 250 | 16 | Azoxystrobin<br>Linuron<br>Carbendazim | 72.1 | 6.4 | [11] |
| 25 | Foods of plant origin and drinks | 126 | 42 | 47 | 18 | Chlorpyrifos<br>Procymidon<br>Primifos-methyl<br>Dimethoate<br>Dieldryna | 33.3 | 38.3 | [39] |

**Table 3.** *Cont.*

| No. | Food Category | No. of Samples | No. of Samples with Detected Residues | No. of Analysed Pesticides | No. of Detected Pesticides | Most Frequently Found Pesticide | % (1) | % (2) | References |
|---|---|---|---|---|---|---|---|---|---|
| 26 | Fruit juices | 106 | 46 | 53 | 9 | Carbendazim<br>Imazalil | 43.4 | 17.0 | [3] |
| 27 | Fruit juices | 21 | 10 | 174 | 21 | Imidacloprid<br>Acetamiprid | 47.6 | 12.0 | [40] |
| 28 | Fruit-based soft drinks | 94 | 85 | 30 | 11 | Carbendazim<br>Imazalil | 90.4 | 36.7 | [4] |
| 29 | Cereals | 89 | 14 | 110 | 3 | Primifos-methyl | 15.7 | 2.7 | [41] |
| 30 | Cereals | 380 | 145 | 292 | Not defined | Permethrin<br>Tebukonazol | 38.0 | - | [23] |
| 31 | Chinese herbal medicines | 294 | 108 | 162 | 42 | Chlorpyrifos | 36.7 | 25.9 | [42] |
| 32 | Plant used in traditional Chinese medicine | 138 | 95 | 116 | 55 | Carbendazim<br>Carbofuran | 68.8 | 47.4 | [43] |
| 33 | Traditional Chinese medicine | 20 | 20 | 55 | 6 | Quintozene Chlorothalonil<br>Chlorpyrifos | 100.0 | 10.9 | [44] |
| 34 | Dried botanical dietary supplements | Not defined | Not defined | 236 | 73 | Carbendazim<br>Metalaxyl<br>Azoxystrobin | - | 30.9 | [45] |
| 35 | Food samples | 31 | 9 | 44 | 8 | Acetamiprid<br>Azoxystrobin | 29.0 | 18.8 | [46] |

(1) The percentage of total number of analysed sample to the total number of detected pesticides. (2) The percentage of detected pesticides to the total number of pesticides analysed.

The literature review revealed the presence of metalaxyl and carbendazim in samples of black pepper, which was also observed in our experiment. In none of the analysed samples of herbs were exceeded levels of concentration (above the MRL) observed, which does not support the results obtained by Reinholds et al. [21] and Kowalska [11], where the concentrations of pesticide residues in 10% [21] of samples of oregano and in 46% [21] and 15% [11] of samples of thyme were above the permissible values. The literature review, in the aspect of the content of pesticide residues in samples of juices, demonstrated that the percentage share of samples in which the sought compounds were identified varied from 43.40% to 90.43% [3,4,40], which is in conformance with the results obtained in this study for the samples of fruit and vegetable juices—50%. In our own study, the most frequently assayed pesticides were acetamiprid, boscalid, clothianidin, and tebucanozole (Table 2), while in the literature reports—acetamiprid, carbendazim, and imazalil (Table 3). In the analysed samples of cereals, no presence of pesticide residues was found. Literature data concerning studies on pesticide residues in cereals in Poland in the years 2009–2013 report the presence of those compounds in the range from 15.73% to 38% of the analysed samples [23,41]. In the presented study, only 3 cereal samples were analysed, which constituted as little as 1.9% of the total number of analysed samples, and that number did not constitute a representative value in relation to the remaining kinds of samples. Summing up the results obtained in this study, it should be emphasised that 51.9% of the samples of plant materials and food products originating from the eastern part of Poland contained pesticide residues, but their levels did not exceed the higher permissible concentrations. Most frequently, pesticide residues were detected in fruit samples (66.7%), compared to the remaining groups of analysed products, where the percentage share of samples containing the sought compounds was at the level of approximately 50% in each group. Special note should be taken of the possible contamination with thiacloprid and trifloxystrobin in fruits of blackcurrant, carbendazim in apples, and azoxystrobin and fludioxonil in strawberries. The analysed samples of fruits contained the largest number and diversity of identified pesticide residues, compared to the remaining samples, which raises concern relating to the quality of those food components. Pesticide cocktails found in food pose a serious threat to people and the environment. Mixtures of pesticides can have far more harmful effects than exposure to individual chemicals, both in humans and other species, such as insects, fish, and birds [47,48]. Pesticides are found in millions of different combinations at different concentrations in our food and landscape. It is probably impossible to create a system sufficiently advanced to be able to assess the full spectrum of health and environmental effects resulting from long-term exposure to hundreds of different pesticides. The results of this study emphasise the importance of monitoring of pesticide residues in herbs and spices, especially in the case of thyme and black pepper, which were identified as the most contaminated matrices in that group of products, in which the percentage share of samples containing pesticide residues was at the level of 80% and 44%, respectively.

## 5. Conclusions

Studies in the area of analysis of pesticide residues are highly important in the estimation of quality of raw materials of plant origin, as well as food. The results obtained in this study indicate that the occurrence of pesticide residues in the analysed products cannot be considered to be a serious threat to human and animal health. Nevertheless, constant monitoring of the content of pesticide residues and strict regulations concerning the highest permissible concentrations of those compounds in food samples are of key importance for the alleviation of potential risk to the health and life of consumers. Due to the harmful effects of the cocktail effect of pesticides, perhaps the only way to minimise the risks to health and the environment is to significantly reduce the overall use of pesticides. There is also a need to introduce urgently needed measures to support farmers to significantly reduce pesticide use and switch to organic farming systems in which synthetic pesticides are replaced by botanical pesticides or chemical control is completely avoided.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2077-0472/10/6/192/s1, Table S1: List of pesticides determined in the positive ionization mode, Table S2: List of pesticides determined in the negative ionization mode.

**Author Contributions:** G.K. and R.K. conceived the research idea and experimental protocol; G.K. coordinated the research; G.K. and R.K. wrote the manuscript; G.K. and R.K. managed writing—review and editing; G.K., U.P., and R.K. had the supervision task; G.K., U.P., and R.K. were involved in crop management and performed the determinations of biochemical and physiological analyses; G.K. managed the data statistical processing; G.K., U.P., and R.K. were involved in bibliographic search. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was financed by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Agrobioengineering of University of Life Sciences in Lublin and for the Faculty of Food Science and Biotechnology of University of Life Sciences in Lublin.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest to disclose.

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**Food Science and Technology Letters**
ISSN: 0976-982X & E-ISSN: 0976-9838, Volume 4, Issue 1, 2013, pp.-41-45.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000214

# MONITORING OF PESTICIDE RESIDUES IN VEGETABLES COLLECTED FROM MARKETS OF SINDH, PAKISTAN

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Received: July 22, 2013; Accepted: August 29, 2013

**Abstract-** Pesticide residues were determined in marketed samples of different vegetables. Samples were collected from different markets of southern Sindh during 2012-13. Residues were extracted with QuEChERS method and were analyzed with Gas Chromatography (GC) coupled with Mass Spectrometry Detector. However, emamectin benzoate and imidacloprid were analyzed with High Performance Liquid Chromatography (HPLC) coupled with Ultraviolet (UV) detector. The insecticide residues in vegetable samples were quantified by using the standards.

Results showed that seven vegetables namely okra, bitter gourd, brinjal, tomato, onion, cauliflower, and chilies, were heavily contaminated with chlorpyrifos, profenofos, endosulfan, imidacloprid, emamectin benzoate, lufenuron, bifenthrin, diafenthiuron, and cypermethrin. Moreover, every vegetable was contaminated with more than one pesticide and majority of samples violated the Japanese MRLs.

Desi spinach, lettuce, bottle gourd, fenugreek, peas, and cluster bean are not sprayed with pesticides normally, but these were found contaminated with trace level residues within MRL(s). This could be due to contaminated soil (from previous crops) and/or may be due to vegetable vendors' mishandling as they use the same water for washing vegetables in series which is a common practice all over Sindh.

Present study recommends that vegetables may be washed thoroughly prior to use and water may be changed after each vegetable wash or washing of vegetables may be done under running tap water in order to minimize pesticide contamination .

**Keywords-** Vegetables, pesticides, residues, QuEChERS, GC-MS, HPLC-UV.

**Citation:** Sheikh S.A., et al. (2013) Monitoring of Pesticide Residues in Vegetables Collected from Markets of Sindh, Pakistan. Food Science and Technology Letters, ISSN: 0976-982X & E-ISSN: 0976-9838, Volume 4, Issue 1, pp.-41-45.



## Introduction

In modern agricultural practices, the use of pesticides provides unquestionable benefits by increasing the production of crops. However, it has the drawback of pesticide residues which remain on the vegetables, constituting potential health risks to consumers. This on one hand leads to violation of the established legal directives to control their levels within the maximum residue levels (MRLs) and simultaneously continue to search for pesticides, which are less persistent and less toxic for human beings on the other [1].

Wide range of pesticides are used for crop protection globally during the cultivation of vegetables due to heavy pest infestation throughout the crop season [2], Literature reveals that vegetables contaminated with pesticide residues above their respective maximum residue limit MRL [3] may pose health hazards to consumers [4-5].

Pesticide application is an essential component of modern crop production technology. Their use has been contentiously increasing over the past decades. In Pakistan, the pesticides application is at maximum on cotton crop followed by fruits and vegetables. Insecticides, herbicides and fungicides are commonly used for crop protection throughout the country [6]. But the overdose of pesticides makes the residue problem, which might pollute our food and be harmful for our health. It has been reported that some of the pesticides are being used in the country where no pre-harvest time frame after last application is maintained [7]. As a result of indiscriminate use of pesticides by the unskilled persons, only a small portion of applied pesticides reaches the targeted species; remainder enters in food chain and is indirectly passed on to human beings. Amongst food items, fresh fruits are the most vulnerable part of the diet, as they are mostly consumed directly after picking as compared to vegetables and grains that are cooked which in turn reduces and metabolizes the pesticide residues [8].

Indiscriminate use of pesticides is very alarming in Pakistan, and they are used in excessive quantities which makes a major food safety concern of consumers and government. Hence, monitoring and assessment of pesticide contamination in farm produce has become a necessity. Particularly, there is need to determine, quantify and confirm pesticide residues in vegetables for both research and regulatory purposes. The pesticides are generally analyzed by spectrophotometry [9-10], thin layer chromatography (TLC) [11-13], high performance liquid chromatography (HPLC) and high performance liquid chromatography-mass spectrophotometry (HPLC-MS) [14-16], gas chromatography (GC) [17-20] and GC-MS [21].

There are many vegetables grown in Sindh province some are sprayed with pesticides (non-organic vegetables) that are more prone to insects pests while some are not sprayed (organic vegetables). In this paper we have studied six organic vegetables viz. lettuce, fenugreek, bottle gourd, peas, cluster beans, and desi spinach and seven non-organic vegetables viz. okra, bitter gourd, brinjal, tomato, onion, cauliflower and chilies for determination of pesticide residues. Therefore, the purpose of the present study is to assess the multi-pesticide residues assessability through Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) methods [22-23] from collected vegetables of the local markets of Southern Sindh.

## Materials and Methods

### Chemicals and Solvents

QuEChERS kits were purchased from the Company Restek Corporation USA. Sodium sulphate was procured from Merck India. Acetonitrile (HPLC grade) used in this study, was obtained from Scharlau Company (Scharlau chemie S.A. La Jota, Barcelona Spain) and were glass distilled before use. Pesticide Standards were supplied by M/S Ali Akbar Group (Pvt.), Ltd. Hyderabad. Silica gel 60 was purchased from Sigma-Aldrich.

### Sampling

Thirteen different vegetables were selected for analysis were okra, bitter gourd, brinjal, tomato, onion, cauliflower, chilies, lettuce, bottle gourd, fenugreek, peas, cluster bean and desi spinach. Twenty samples of each vegetable were collected from various vegetable shops of Jamshoro and Tando Mohammad Khan metropolis, packed in polyethylene bags; labeled and transported in ice preserved packs to the laboratory of Institute of Food Sciences and Technology Sindh Agriculture University Tandojam. The samples were kept in freezer at -20°C till extraction.

### Extraction

The QuEChERS sample preparation method for pesticides (AOAC Official Method 2007.01) was applied to all the samples. The sample was homogenized by Homogenizer by the addition of 1g sodium sulfate and 20ml of acetonitrile and 1g salt mixture. The homogenized sample was transferred to 50ml tube shaken vigorously for 3 min and centrifuged at 4000 rpm for 5 min. The supernatant (5ml) were transferred to a 15 ml PTFE tube to which 750 mg $MgSO_4$ and 250 mg PSA were added. The extract was shaken using a vortex mixer for 1 min and centrifuged at 4000 rpm again for 5 min. Supernatant was then filtered through a 0.45 mm PTFE filter (13 mm diameter) and transferred to 10ml vials and sealed for quantification using gas chromatograph equipped with mass spectrometry (GC-MS).

### Analytical Technique

For analysis, Agilent (6890N) gas chromatograph system equipped with a model 7673 auto-sampler Mass Spectroscopy (GC/MS) was used. Residues were separated through Agilent DB-1 capillary column (30 m X 0.2 5mm with 0.1 µm film) with nitrogen flow rate 30 ml / minute, air flow rate 60 ml/minute was used under the following conditions.

The inlet was set at 250°C and the MS source at 250°C. The oven was programmed: 40°C, 1.5 min., 15°C/min., 150°C; 7°C/min., 225°C; 25°C/min., 290°C, 15 min with a constant column flow rate of 1 mL/min.

High Performance Liquid chromatography coupled with ultra violet (UV) detector was used for imdacloprid and emamectin benzoate residues determination [Table-1]. Separation was carried out on a Supelco LC-18 column (250mm× 4.6mm ID, 5µm) (Supelco Park, Bellefonte, USA). The mobile phase was acetonitrile and de-ionized water.

*Table 1- HPLC parameters for determination of HPLC amenable pesticides (emamectin benzoate and imidacloprid residues).*

| Imidacloprid | Emamectin Benzoate |
|---|---|
| Flow rate = 1.2ml/min | Flow rate = 1.2ml/min |
| Ratio: Acetonitrile : Water (de-ionized) 35:65 | Ratio: Acetonitrile : Water (de-ionized) 98:02:00 |
| Wavelength = 270nm | Wavelength =246nm |
| Injection volume = 20µl | Injection volume =30 µl |

## Results and Discussion

Pesticides are widely used to increase the productivity of agricultural commodities and hence are essential component in modern agriculture. These chemicals are actually produced and/ or developed for agriculture pest control. Pesticides spray on vegetable crops is very common practice which not only kills insect/ pests but also stuck/ get inside the vegetables through minute pores thereby becoming its component. These are called pesticide residues that remain on the surface or inside of the vegetables and may become a great health hazard after consumption. Contamination of vegetables result from pesticide spray, as well as from improper handling, contaminated environment (air, soil or water) and from cross contamination processes.

The present study was undertaken to evaluate the pesticide residues from market samples of Southern Sindh, the pesticide compounds in collected vegetable samples were identified by comparing their retention time with respect to their technical grade reference standards.

In the present research work an attempt has been made to estimate multi-residues via QuEChERS method, the results indicate the presence of pesticide residues in different vegetables. Vegetable samples were analyzed in triplicate for the presence of pesticides residues [Table-2] and [Table-3].

Twenty samples of each six vegetable, that is, lettuce, fenugreek, bottle gourd, peas, cluster beans, and desi spinach which are usually organically grown (without pesticide spray) were collected for pesticide residue analysis. It was observed that all the six vegetables were found contaminated with trace amount of pesticide residues. Lettuce was found contaminated with trace amounts of endosulfan (0.05ppm), imidacloprid (0.11ppm) and bifenthrin (0.2ppm). Fenugreek was contaminated with endosulfan (0.13ppm), imidacloprid (0.11ppm) and bifenthrin (0.013ppm). Bottle gourd contained the residues of chlorpyrifos (0.005ppm), endosulfan (0.07ppm), imidacloprid (0.21ppm) and bifenthrin (0.05ppm).

Peas had chlorpyrifos (0.01ppm), endosulfan (0.09ppm), imidacloprid (0.13ppm) and bifenthrin (0.01ppm). Cluster beans contained endosulfan (0.04ppm), imidacloprid (0.31ppm) and bifenthrin (0.01ppm) and desi spinach had residues of chlorpyrifos (0.003ppm), endosulfan (1.02ppm), imidacloprid (0.65ppm) and bifenthrin (0.07ppm).

Percent contamination and maximum residues were also calculated for each vegetable and results revealed that percent contamination in lettuce of endosulfan was 10% (0.1 max residues), imidacloprid

35% (0.51max residues) and bifenthrin was 30% (0.45 max residues). In fenugreek endosulfan was 25% (0.22 max residues), imidacloprid 35% (0.19 max residues) and bifenthrin was 30% (0.42 max residues). In bottle gourd chlorpyrifos 40% (0.008 max residues), endosulfan 35% (0.13 max residues), imidacloprid 30% (0.49 max residues) and bifenthrin was 35% (0.15 max residues). In peas chlorpyrifos 30% (0.045 max residues), endosulfan 25% (0.17 max residues), imidacloprid 25% (0.34 max residues) and bifenthrin was 20% (0.04 max residues). In cluster beans endosulfan 15% (0.05 max residues), imidacloprid 20% (0.8 max residues) and bifenthrin was 10% (0.03 max residues) and in desi Spinach chlorpyrifos 25% (0.007 max residues), endosulfan 55% (1.28 max residues), imidacloprid 35% (1.2 max residues) and bifenthrin was 35% (0.13 max residues).

Although these vegetables are organically grown but our findings [Table-2] showed that these vegetables had trace amount of residues which may be due to the reason that the retailers contaminate these vegetables by washing with same water which they use for washing heavily sprayed vegetables. The other possible reason for contamination of organic vegetables may be growing these vegetables on the soil contaminated from previous crop. This is also in conformity with Hill [25] who proposed that the fruits are usually mixed in the lots in trade, the residue data from these composite samples were therefore, potentially misleading.

*Table 2- Pesticide residues (ppm) in organic vegetables*

| Pesticides | Parameter | Lettuce | Fenugreek | Bottlegourd | Peas | Cluster Beans | Desi Spinach |
|---|---|---|---|---|---|---|---|
| chlorpyrifos | Residue found | ND | ND | 0.005 | 0.01 | ND | 0.003 |
| | mrl | ND | ND | 0.01 | 0.05 | ND | 0.01 |
| | % + ve (%violate) | ND | ND | 40- ND | 30- ND | ND | 25- ND |
| | Max - min | ND | ND | 0.008- ND | 0.045- ND | ND | 0.007- ND |
| Endosulfan | Residue found | 0.05 | 0.13 | 0.07 | 0.09 | 0.04 | 1.02 |
| | mrl | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 2 |
| | % + ve (%violate) | 10- ND | 25- ND | 35- ND | 25- ND | 15- ND | 55- ND |
| | Max - min | 0.1- ND | 0.22- ND | 0.13- ND | 0.17- ND | 0.05- ND | 1.28- ND |
| Imidacloprid | Residue found | 0.11 | 0.06 | 0.21 | 0.13 | 0.31 | 0.65 |
| | mrl | 3 | 5 | 1 | 3 | 3 | 15 |
| | % + ve (%violate) | 35-ND | 35--ND | 30- ND | 25- ND | 20- ND | 35- ND |
| | Max - min | 0.51- ND | 0.19- ND | 0.49- ND | 0.34- ND | 0.8- ND | 1.2- ND |
| Bifenthrin | Residue found | 0.2 | 0.013 | 0.05 | 0.01 | 0.01 | 0.07 |
| | mrl | 3 | 2 | 0.4 | 0.05 | 0.2 | 0.2 |
| | % + ve (%violate) | 30-ND | 30-ND | 35-ND | 20-ND | 10-ND | 35-ND |
| | Max- min | 0.45-ND | 0.42-ND | 0.15-ND | 0.04-ND | 0.03-ND | 0.13-ND |

*() = MRLs ppm; ND = Non Detected Note: 20 samples were analyzed for each vegetable*

*For each pesticide 1st row shows residue detected in ppm, 2nd row shows Japanese MRL[1], 3rd row shows percent samples found positive, 4th row shows percent samples violating MRL.*

Similarly, twenty samples of each seven non-organic vegetables namely okra, bitter gourd, brinjal, tomato, onion, cauliflower and chilies were also purchased from markets for pesticide residues determination. [Table-3] shows that nine pesticides, that is, chlorpyrifos, profenofos, endosulfan, imidacloprid, emamectin benzoate, lufenuron, bifenthrin, diafenthiuron and cypermethrin, which are most commonly used on different vegetables, were detected and majority of them were found with residues above their respective MRLs. Results [Table-3] revealed that okra contained the residues of chlorpyrifos (0.09ppm), endosulfan (0.93ppm), imidacloprid (0.29ppm) and bifenthrin (0.031ppm). Bitter gourd was found with residues of endosulfan (0.28ppm), imidacloprid (0.38ppm), emamectin benzoate (0.06ppm), diafenthiuron (0.015ppm) and cypermethrin (0.022ppm). Brinjal was contaminated with endosulfan (0.33ppm), imidacloprid (0.74ppm), emamectin benzoate (0.04ppm) and diafenthiuron (0.017ppm). Tomato had residues of endosulfan (0.35ppm), imidacloprid (0.82ppm), bifenthrin (0.26ppm) and diafenthiuron (0.008ppm). Onion was contaminated with profenofos (0.046ppm), endosulfan (0.17ppm) and imidacloprid (0.15ppm). Cauliflower was found contaminated with chlorpyrifos (0.04ppm), endosulfan (0.25ppm), imidacloprid (0.25ppm), emamectin benzoate (0.11ppm) and cypermethrin (0.58ppm). Chilies were contaminated with endosulfan (0.22ppm), emamectin benzoate (0.07ppm), lufenuron (0.37ppm) and cypermethrin (0.29ppm).

Percent contamination ratio and max residues with percent violated samples were also determined of each vegetable. It was noted that contamination of okra was 60% with chlorpyrifos and 25% samples violated the MRLs (0.13 max residues), 55% with endosulfan and 25% samples violated the MRLs (1.5 max residues), 35% with imidacloprid and 25% samples violated the MRLs (0.74 max residues) and 35% with bifenthrin and 25% samples violated the MRLs (0.098 max residues). In the case of bitter gourd 35% contained endosulfan and 15% samples violated the MRLs (0.57 max residues), 45% with imidacloprid and 20% samples violated the MRLs (1.32 max residues), 35% with emamectin benzoate and 10% samples violated the MRLs (0.14 max residues), 56% with diafenthiuron and 30% samples violated the MRLs (0.05 max residues) and 30% in cypermethrin with no samples violated the MRLs (0.08 max residues). Similarly, for brinjal 40% with endosulfan and 15% samples violated the MRLs (0.59 max residues), 30% with imidacloprid and 15% samples violated the MRLs (2.65 max residues), 15% with emamectin benzoate with no samples violated the MRLs (0.07 max residues), and 45% with diafenthiuron and 35% samples violated the MRLs (0.03 max residues). 25% tomato samples had endosulfan and 10% violated the MRLs (0.68 max residues), 40% had imidacloprid and 10% violated the MRLs (2.12 max residues), 30% had bifenthrin and 15% violated the MRLs (0.66 max residues) and 10% had diafenthiuron and 10% violated the MRLs (0.03 max residues).

Onion grows underground and is prone to accumulate more pesticide residues and 20% samples were polluted with profenofos and 10% violated the MRLs (0.07 max residues), 35% with endosulfan and 10% violated the MRLs (0.42 max residues), 35% with imidacloprid and 10% violated the MRLs (0.5 max residues). Cauli-

flower whose leaves have waxy layer to roll of spray droplets and its 25% samples had chlorpyrifos and 10% violated the MRLs (0.06 max residues), 35% had endosulfan and 15% violated the MRLs (0.53 max residues), 45% had imidacloprid and 15% violated the MRLs (0.55 max residues), 30% had emamectin benzoate and 10% violated the MRLs (0.92 max residues) and 25% had cypermethrin and 10% violated the MRLs (1.67 max residues; whereas 45% chilies samples were contaminated with endosulfan and 15% violated the MRLs (0.57 max residues), 25% with emamectin benzoate and 5% violated the MRLs (0.28 max residues), 35% with lufenuron and 10% violated the MRLs (0.67 max residues) and 35% with cypermethrin and 20% violated MRLs (0.94 max residues).

Out of 13 different vegetable samples collected from Jamshoro and Tando Muhammad Khan markets, it was noted that organochlorine, organophosphate, nicotinoid and pyrethroid pesticides are most commonly used in Sindh and found frequently in vegetable samples collected from markets with the exception of lufenuron (insect growth regulator) which was found only in brinjal, tomato, chilies and lettuce.

Majority of the samples violated MRLs and these results are in conformity with the findings of earlier study [24] in which fruit samples of Karachi market were taken and most of the samples were found contaminated with multiple pesticide residues.

The persistent nature of different pesticides, mishandling, environmental pollution and presence of pesticide residues in vegetables has now become a global concern. Organophosphorous, organochlorine and nicotinoid pesticides, along with mixture of different pesticides in fruits and vegetables were also reported all over the world by many researchers [26-34].

*Table 3- Pesticide residues (ppm) in non-organic vegetables*

| Pesticides | Parameters | Okra | Bittergourd | Brinjal | Tomato | Onion | Cauliflower | Chilies |
|---|---|---|---|---|---|---|---|---|
| Chlorpyrifos | Residues Found | 0.09 | ND | ND | ND | ND | 0.04 | ND |
| | mrl | 0.1 | ND | ND | ND | ND | 0.05 | ND |
| | % + ve (%violat) | 60 (25) | ND | ND | ND | ND | 25(10) | ND |
| | Max - min | 0.13- ND | ND | ND | ND | ND | 0.06-ND | ND |
| Profenofos | Residues Found | ND | ND | ND | ND | 0.046 | ND | ND |
| | mrl | ND | ND | ND | ND | 0.05 | ND | ND |
| | % + ve (%violat) | ND | ND | ND | ND | 20(10) | ND | ND |
| | Max-min | ND | ND | ND | ND | 0.07-ND | ND | ND |
| Enosulfan | Residues Found | 0.93 | 0.28 | 0.33 | 0.35 | 0.17 | 0.25 | 0.22 |
| | mrl | 1 | 0.5 | 0.5 | 0.5 | 0.2 | 0.5 | 0.5 |
| | % + ve (%violat) | 55(25) | 35(15) | 40(15) | 25(10) | 35(10) | 35(15) | 45(15) |
| | Max-min | 1.5-ND | 0.57-ND | 0.59-ND | 0.68-ND | 0.42-ND | 0.53-ND | 0.57-ND |
| Imidacloprid | Residues Found | 0.29 | 0.38 | 0.74 | 0.82 | 0.15 | 0.25 | ND |
| | mrl | 0.5 | 1 | 2 | 2 | 0.2 | 0.4 | ND |
| | % + ve (%violat) | 35(25) | 45(20) | 30(15) | 40(10) | 35(10) | 45(15) | ND |
| | Max-min | 0.74-ND | 1.32-ND | 2.65-ND | 2.12-ND | 0.5-ND | 0.55-ND | ND |
| Emamectin Benzoate | Residues Found | ND | 0.06 | 0.04 | ND | ND | 0.11 | 0.07 |
| | mrl | ND | 0.1 | 0.1 | ND | ND | 0.5 | 0.2 |
| | % + ve (%violat) | ND | 35(10) | 15-ND | ND | ND | 30(10) | 25(5) |
| | Max-min | ND | 0.14-ND | 0.07-ND | ND | ND | 0.92-ND | 0.28-ND |
| Lufenuron | Residues Found | ND | ND | ND | ND | ND | ND | 0.37 |
| | mrl | ND | ND | ND | ND | ND | ND | 0.5 |
| | % + ve (%violat) | ND | ND | ND | ND | ND | ND | 35(10) |
| | Max-min | ND | ND | ND | ND | ND | ND | 0.67-ND |
| Bifenthrin | Residues Found | 0.031 | ND | ND | 0.26 | ND | ND | ND |
| | mrl | 0.04 | ND | ND | 0.5 | ND | ND | ND |
| | % + ve (%violat) | 35(25) | ND | ND | 30(15) | ND | ND | ND |
| | Max-min | 0.098-ND | ND | ND | 0.66-ND | ND | ND | ND |
| Diafenthiuron | Residues Found | ND | 0.015 | 0.017 | 0.008 | ND | ND | ND |
| | mrl | ND | 0.02 | 0.02 | 0.01 | ND | ND | ND |
| | % + ve (%violat) | ND | 56(30) | 45(35) | 10(10) | ND | ND | ND |
| | Max-min | ND | 0.05-ND | 0.03-ND | 0.03-ND | ND | ND | ND |
| Cypermethrin | Residues Found | ND | 0.022 | ND | ND | ND | 0.58 | 0.29 |
| | mrl | ND | 0.05 | ND | ND | ND | 1 | 0.5 |
| | % + ve (%violat) | ND | 30-ND | ND | ND | ND | 25(10) | 35(20) |
| | Max-min | ND | 0.08-ND | ND | ND | ND | 1.67-ND | 0.94-ND |

*() = MRLs ppm; ND = Non Detected; Note: 10 samples were analyzed for each vegetable*

*For each pesticide 1st row shows residue detected in ppm, 2nd row shows Japanese MRL[1], 3rd row shows percent samples found positive, 4th row shows percent samples violating MRL.*

Organochlorine (Endosulfan) pesticide is banned due to its highly toxic and persistent nature but unfortunately it is still used by local farmers of Sindh. Results [Tables-2] and [Table-3] showed that all the 13 vegetable samples were contaminated with toxicant endosulfan. Similarly, organophosphate poisoning cases are also global health problem with annually 0.2 million deaths, because of their direct effect on Central Nervous System, cardiovascular system and reproductive system [35]. Chiron, et al [36] also reported the toxic effects of carbamates and declared them as potential environmental pollutants.

The observations of different compounds [Table-2] and [Table-3] were compared with recommended Maximum residue limits (MRLs). The comparison of results with their respective MRLs have led to an insight which suggests that majority of vegetables had residual levels far above the MRLs, hence were unfit for human consumption. It was further observed that same water was being used for washing of different vegetables which increased the contamination ratio.

**Conclusion**

It was concluded that pesticide spray is most common practice in Lower Sindh and single vegetable was found with more than one pesticide with residual level above mentioned MRLs. Present study recommends that vegetable may be thoroughly washed prior to use and water may be changed after each vegetable wash or washing of vegetables may be done under running tap water in order to minimize pesticide contamination ratio. The study further recommends that.

**Conflict of Interest :** None Declared

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FULLERENE Journal of Chemistry

Fullerene Journ. Of Chem Vol.3 No.2: 63-69, 2018
ISSN 2598-1269

# Analisis Residu Pestisida dalam Tomat, Cabai Rawit dan Wortel dari Beberapa Pasar Tradisional di Sulawesi Utara

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INFO ARTIKEL

Diterima 16 Oktober 2018
Disetujui 30 Oktober 2018

***Keywords:***
*chlorpyrifos,*
*hplc,*
*tomato,*
*Cayenne pepper,*
*carrot*

***Kata kunci:***
*klorpirifos,*
*hplc,*
*tomat,*
*cabai rawit,*
*wortel*

ABSTRACT

*One method to eradicate plant pests carried out by farmers in the North Sulawesi area is to use pesticides because they are considered easy to obtain, the price is still affordable, and very effective at killing plant pests. However, improper use of pesticides results in the loss of pesticide residues in plants which causes environmental pollution, health problems in humans and inhibits trade. Therefore, it is necessary to monitor the use of pesticides through the fulfillment of the maximum residual limit (BMR). This study aims to analyze pesticide residues in tomatoes, cayenne pepper, and carrots using the method of High Performance Liquid Chromatography (HPLC) which was previously optimized and validated. The research samples were tomatoes, cayenne pepper, and carrots, taken from Pasar Bersehati Tomohon, Pasar Karombasan Manado, and Pasar Kawangkoan Minahasa, then taken to the Chemistry Laboratory of Manado State University to be extracted and analyzed. The results of this study indicate that pesticides with chlorpyrifos active ingredients were detected in almost all samples analyzed, although the levels were still below the specified BMR value, ie 1 mg/kg sample. The highest chlorpyrifos level was found in tomato samples taken from Pasar Kawangkoan Minahasa, which was 0.3150 mg/kg. The results of this study also showed that samples that were washed before extraction caused a decrease in the residual content of the petisides.*

ABSTRAK

Salah satu cara memberantas hama tanaman yang dilakukan oleh para petani di daerah Sulawesi Utara adalah menggunakan pestisida karena dianggap mudah didapat, harganya masih bisa dijangkau, dan sangat efektif membunuh hama tanaman. Namun, penggunaan pestisida yang tidak tepat mengakibatkan tertinggalnya residu pestisida tersebut pada tanaman sehingga menyebabkan pencemaran lingkungan, gangguan kesehatan pada manusia dan menghambat perdagangan. Oleh karena itu, perlu dilakukan pengawasan terhadap penggunaan pestisida melalui pemenuhan nilai batas maksimum residu (BMR). Penelitian ini bertujuan untuk menganalisis residu pestisida dalam tomat, cabai rawit, dan wortel dengan metode *High Performance Liquid Chromatography* (HPLC) yang telah dioptimasi dan divalidasi sebelumnya. Sampel penelitian berupa tomat, cabai rawit, dan wortel, diambil dari Pasar Tomohon, Pasar Karombasan, dan Pasar Kawangkoan, kemudian dibawa ke laboratorium Kimia Universitas Negeri Manado untuk diekstraksi dan dianalisis. Hasil penelitian ini menunjukkan bahwa pestisida dengan bahan aktif klorpirifos terdeteksi hampir pada semua sampel yang dianalisis, walaupun kadarnya masih berada di bawah nilai BMR yang ditetapkan, yaitu 1 mg/kg sampel. Kadar klorpirifos tertinggi dijumpai pada sampel tomat yang diambil dari Pasar Kawangkoan, yakni 0,3150 mg/kg sampel. Hasil penelitian ini juga menunjukkan bahwa sampel yang dicuci terlebih dahulu sebelum diekstraksi menyebabkan terjadinya penurunan kadar residu petisidanya.

**Kata kunci:** *klorpirifos, hplc, tomat, cabai rawit, wortel*

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## PENDAHULUAN

Berdasarkan hasil survey yang kami lakukan terhadap beberapa petani yang ada di daerah Sulawesi Utara, umumnya mereka memberantas hama tanaman dengan menggunakan pestisida karena mudah didapat, harganya relatif masih dapat dijangkau, dan sangat efektif membunuh hama tanaman [1]. Padahal penggunaan pestisida secara tidak tepat dapat meninggalkan residu pestisida tersebut pada tanaman yang dapat menyebabkan pencemaran lingkungan, gangguan kesehatan pada manusia, serta dapat menghambat perdagangan [2]. Oleh karena itu, perlu dilakukan pengawasan terhadap penggunaan pestisida melalui pemenuhan nilai batas maksimum residu (BMR) sehingga dapat menjamin keamanan pangan dengan cara membatasi kadar residu pestisida pada komoditas hasil-hasil pertanian.

Salah satu jenis pestisida yang banyak digunakan petani adalah pestisida golongan organofosfat dengan bahan aktif klorpirifos. Hal ini disebabkan karena pestisida golongan organofosfat memiliki karakteristik yang lebih menguntungkan dibandingkan dengan pestisida organoklorin, seperti mudah terurai dan waktu persistennya yang singkat [2]. Pestisida organofosfat melindungi tanaman dari hama dengan menghambat aktivitas enzim asetilkolineterase pada serangga. Penggunaannya dengan cara disemprot pada tanaman atau tanah, menyebabkan residunya dapat ditemukan di permukaan air tanah, buah-buahan, sayuran dan air minum [3].

Penelitian yang kami lakukan sebelumnya menunjukkan bahwa pestisida dengan bahan aktif klorpirifos terdeteksi pada semua sampel sayur kubis yang diambil dari beberapa pasar tradisional di Sulawesi Utara, walaupun kadarnya masih di bawah nilai BMR yang ditetapkan[1]. Keberadaan pestisida organofosfat dengan bahan aktif klorpirifos dalam produk-produk pertanian juga telah dilaporkan oleh beberapa peneliti di Indonesia. Panggabean [4] dalam penelitiannya tentang analisis residu klorpirifos dalam sayur-sayuran dengan teknik HPLC, menemukan adanya residu klorpirifos dalam sampel sayur kubis yang dianalisis dengan kadar 0,131 ± 0,008 μg/kg. Kadar tersebut masih jauh di bawah nilai BMR yang ditetapkan untuk klorpirifos dalam sayur kubis, yaitu 1 mg/kg (SNI 7313:2008). Marzuki dkk., juga menemukan adanya residu klorpirifos pada sampel uji Sawi Hijau di Kabupaten Gowa, walaupun masih dalam ambang toleransi BMR [5]. Berdasarkan hasil penelitian Triani dkk., rata-rata residu pestisida klorpirifos pada kacang panjang di Kecamatan Baturiti Kecamatan Marga dan Kecamatan Kerambitan masing-masing sebesar 0,0397 mg/kg, 0,0169 mg/kg, dan 0,0118 mg/kg, hasil tersebut masih di bawah BMR, sedangkan dikecamatan Penebel sebesar 0,2447 mg/kg masih berada di atas BMR [6]

Berdasarkan hasil penelusuran literatur maupun pengamatan di lapangan yang kami lakukan sejauh ini, masih sangat kurang penelitian yang dilakukan untuk analisis residu pestisida pada hasil-hasil pertanian di daerah Sulawesi Utara. Oleh karena itu, penelitian ini dilakukan dengan tujuan untuk menentukan kadar residu klorpirifos dalam sampel tomat, cabai rawit, dan wortel diambil dari beberapa pasar tradisional yang ada di Sulwesi Utara menggunakan metode HPLC yang telah dioptimasi dan divalidasi sebelumnya [7].

### Bahan dan Metode

Peralatan yang digunakan dalam penelitian ini antara lain: seperangkat alat HPLC (Agilent 1260 Infinity Binary LC) yang dilengkapi dengan detektor DAD, utosampler, dan kolom Zorbax Eclipse Plus C18 (3,5 μm; 2,1 x 100 mm), Spektrofotometer UV-Vis PerkinElmer Lambda 25, rotavapor, sonikator, neraca analitik, penyaring vakum beserta saringan berpori 0,4 - 0,45 μm, blender, corong pisah dengan tutup, penangas air, pompa vakum, dan alat gelas yang lazim.

Bahan-bahan yang digunakan antara lain sampel buah tomat, cabai rawit, dan wortel yang diambil dari Pasar Bersehati Tomohon, Pasar Karombasan Manado, dan Pasar Kawangkoan Minahasa, Klorpirifos (Kemurnian ≥ 99,9%, Merck), Asetonitril (gradient grade, ≥ 99,9%, Merck), etilasetat, $Na_2SO_4$ anhidrat, aquabidest.

#### *Prosedur Penelitian*

Secara garis besar, prosedur penelitian ini dibagi menjadi beberapa tahap, yaitu:

1. *Penentuan panjang gelombang serapan maksimum klorpirifos*

Panjang gelombang serapan maksimum klorpirifos ditentukan dengan mengukur serapan larutan baku klorpirifos 10 ppm menggunakan spektrofotometer UV/Vis Lambda 25.

2. *Penentuan kondisi optimum HPLC*

Kondisi optimum HPLC ditentukan menggunakan kolom Zorbax Eclipse Plus C18 (3,5 μm; 2,1 x 100 mm) menggunakan detektor DAD pada panjang gelombang 289 nm. Penentuan kondisi optimum HPLC meliputi:

*a. Penentuan Komposisi Fasa Gerak*

Fasa gerak yang digunakan dalam penelitian ini adalah air dan asetonitril dengan komposisi air : asetonitril divariasikan dari 100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80, 10:90, dan 0:100. Sebanyak 5 μL larutan baku klorpirifos 2 ppm diinjeksikan ke dalam kolom HPLC kemudian ditentukan waktu retensi dan luas area puncak kromatogram masing-masing komposisi fasa gerak tersebut.

*b. Penentuan Laju Alir Fasa Gerak*

Laju alir fasa gerak dipalajari dengan mengukur waktu retensi dan luas area puncak kromatogram larutan baku klorpirifos 2 ppm pada laju alir yang divariasikan dari 0,3 – 0,9 mL/menit menggunakan komposisi fasa gerak optimum yang telah ditentukan sebelumnya.

*c. Penentuan Volume Injeksi*

Volume injeksi sampel dipelajari dengan mengukur luas area puncak kromatogram larutan baku klorpirifos 2 ppm dengan volume injeksi divariasikan dari 5 – 30 μL menggunakan komposisi dan laju alir fasa gerak optimum yang telah ditentukan sebelumnya.

3. *Penentuan Kinerja Analitik*

Setelah ditentukan kondisi optimum HPLC, selanjutnya ditentukan kinerja analitik yang meliputi:

*a. Presisi*

Presisi ditetapkan berdasarkan keterulangan (*repeatability*) yang ditentukan dengan mengukur luas area puncak kromatogram larutan baku klorpirifos 2 ppm pada kondisi optimum HPLC. Pengukuran diulangi sebanyak 7 kali kemudian ditentukan simpangan baku relatif (SBR) atau keoefisien variasi (KV).

*b. Linearitas dan Kurva kalibrasi*

Kurva kalibrasi dibuat dengan mengukur luas area puncak kromatogram larutan baku klorpirifos dengan konsentrasi yang divariasikan dari 0 – 10 ppm menggunakan kondisi optimum HPLC yang telah ditentukan sebelumnya. Kemudian dibuat kurva kalibrasi dengan mengalurkan luas area puncak kromatogram (sebagai sumbu-y) terhadap konsentrasi larutan baku klorpirifos (sebagai sumbu-x), lalu dihitung persamaan garis regresi dan koefisien korelasinya.

*c. Batas Deteksi dan Batas Kuntitasi*

Dalam penelitian ini, nilai batas deteksi (LOD) dan batas kuantitasi (LOQ) dihitung secara statistik melalui garis regresi dari kurva kalibrasi [8]. Nilai LOD dihitung menggunakan persamaan:

$$LOD = \frac{3\,S_{(y/x)}}{b} \quad (1)$$

Sedangkan nilai LOQ dihitung menggunakan persamaan:

$$LOQ = \frac{10\,S_{(y/x)}}{b} \quad (2)$$

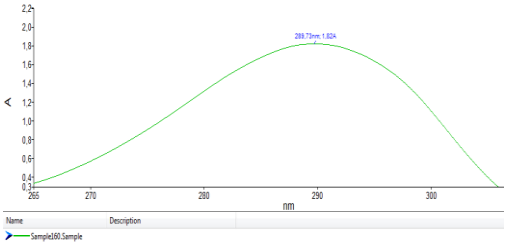
Dimana $S_{(y/x)}$ adalah simpangan baku residual, dan $b$ adalah slope dari persamaan garis regresi linier.

## Hasil dan Pembahasan

1. *Panjang Gelombang Serapan Maksimum Klorpirifos*

Panjang gelombang serapan maksimum klorpirifos ditentukan dengan mengukur serapan larutan baku klorpirifos 10 mg/L menggunakan spektrofotometer UV/Vis Lambda 25 pada rentang panjang gelombang 190 – 700 nm. Berdasarkan hasil pengukuran diperoleh panjang

gelombang serapan maksimum klorpirifos adalah $\lambda$ = 289 nm (Gambar 1).




**Gambar 1.** Panjang gelombang serapan klorpirifos

2. *Penentuan Kondisi Optimum HPLC*

a. *Pengaruh Komposisi Fasa gerak*

Fasa gerak yang digunakan dalam penelitian ini adalah air dan asetonitril yang bersifat lebih polar dibandingkan fasa diam, yakni C18 yang bersifat nonpolar. Komposisi fasa gerak optimum ditentukan berdasarkan luas area puncak kromatogram dan waktu retensi, karena luas puncak merupakan parameter yang lebih akurat untuk pengukuran kuantitatif [9].

Hasil pengukuran pada berbagai komposisi fasa gerak air:asetonitril diperlihatkan dalam Tabel 1.

**Tabel 1.** Pengaruh komposisi air:asetonitril terhadap waktu retensi dan luas area

| Komposisi Air:Asetonitril | Waktu Retensi (menit) | Luas Area (mAU*s) |
|---|---|---|
| 50 : 50 | 6,877 | 1753,36926 |
| 40 : 60 | 6,724 | 32,82681 |
| 30 : 70 | 3,055 | 32,73163 |
| 20 : 80 | 1,797 | 32,44448 |
| 10 : 90 | 1,184 | 32,23305 |
| 0 : 100 | 0,999 | 32,98140 |

Berdasarkan data pada Tabel 1 dapat dilihat bahwa jika komposisi astonitril diperbesar, maka luas area puncak kromatogram klorpirifos semakin berkurang namun waktu retensinya semakin singkat. Luas area puncak kromatogram klorpirifos terbesar terbaca pada komposisi air:asetonitril (50:50), namun waktu retensinya lebih lama dan kromatogram yang dihasilkan tidak simetris. Oleh karena itu, komposisi fasa gerak air : asetonitril yang digunakan dalam penelitian ini adalah 10 : 90, karena waktu pengukurannya lebih singkat dan luas area puncak kromatogramnya masih cukup baik.

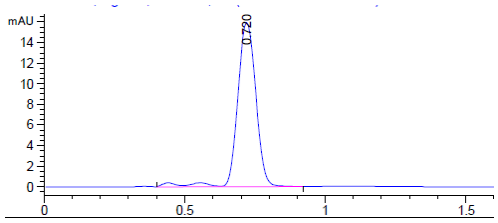
b. *Pengaruh Laju Alir Fasa Gerak*

Pengukuran laju alir fasa gerak dilakukan menggunakan komposisi fasa gerak optimum yang telah ditentukan sebelumnya, yaitu pada perbandingan air : asetonitril 10 : 90. Hasil pengkuran tersebut ditunjukkan dalam Tabel 2 berikut.

**Tabel 2.** Pengaruh Laju Alir Fasa Gerak terhadap Waktu Retensi dan Luas Area

| Laju Alir Fasa Gerak (mL/menit) | Waktu Retensi (menit) | Luas Area (mAU*s) |
|---|---|---|
| 0,30 | 1,682 | 162,69608 |
| 0,50 | 1,006 | 97,39092 |
| 0,70 | 0,720 | 72,81155 |
| 0,90 | 0,561 | 61,86494 |

Berdasarkan data pada Tabel 2, dapat dilihat bahwa semakin tinggi laju alir fasa gerak, semakin singkat waktu retensi dan semakin kecil luas area puncak kromatogram yang dihasilkan. Kromatogram terbaik dengan waktu retensi lebih singkat diperoleh pada laju alir 0,70 mL/menit (Gambar 2). Pada laju alir 0,90 mL/menit, kromatogram yang dihasilkan tidak simetris lagi.




**Gambar 2.** Kromatogram klorpirifos pada laju alir fasa gerak 0,70 mL/menit

c. *Pengaruh Volume Injeksi Sampel*

Pengukuran parameter volume injeksi sampel dilakukan menggunakan komposisi dan laju alir fasa gerak optimum yang telah ditentukan sebelumnya, yaitu komposisi air : asetonitril 10:90 dan laju alir 0,70 mL/menit. Hasil pengkuran tersebut ditunjukkan dalam Tabel 3 berikut.

**Tabel 3.** Pengaruh Volume Injeksi Sampel terhadap Waktu Retensi dan Luas Area

| Volume | Waktu | Luas Area |
|---|---|---|

| Injeksi Sampel (μL) | Retensi (menit) | (mAU*s) |
|---|---|---|
| 5,0 | 1,184 | 32,23305 |
| 10,0 | 1,008 | 65,02857 |
| 15,0 | 1,008 | 97,40105 |
| 20,0 | 1,004 | 129,75168 |
| 25,0 | 1,001 | 178,27931 |
| 30,0 | 0,993 | 210,57770 |

Berdasarkan data pada Tabel 3 dapat dilihat bahwa luas area puncak kromatogram larutan standar klorpirifos semakin besar jika volume injeksi sampel diperbesar, namun pada volume injeksi ≥ 20 μL, kromatogram larutan baku klorpirifos menjadi tidak simetri lagi. Oleh karena itu, dalam penelitian ini digunakan volume injeksi sampel 15 μL, karena menghasilkan kromatogram yang lebih baik.

Berdasarkan hasil otimasi parameter-parameter kromatografik di atas, maka dapat diringkaskan kondisi optimum HPLC untuk penetapan residu klorpirifos seperti ditunjukkan dalam Tabel 4 berikut.

**Tabel 4.** Kondisi optimum HPLC untuk penentuan klorpirifos

| Parameter | Hasil Pengukuran |
|---|---|
| Kolom | Zorbax Eclipse Plus C18 (3,5 μm; 2,1 x 100 mm) |
| Detector | DAD |
| Panjang gelombang | 289 nm |
| Komposisi fasa gerak | Air : Asetonitril (10 : 90) |
| Laju alir fasa gerak | 0,70 mL/menit |
| Volume injeksi sampel | 15 μL |

*3. Penentuan Kinerja Analitik*

*a. Presisi*

Presisi merupakan ukuran yang menunjukkan derajat kesesuaian antara hasil uji individual, diukur melalui penyebaran hasil individual dari rata-rata jika prosedur diterapkan secara berulang pada sampel-sampel yang diambil dari campuran yang homogen [8]. Pada penelitian ini, presisi ditetapkan berdasarkan keterulangan luas area kromatogram hasil analisa dengan 7 kali pengulangan pada larutan standar. Kriteria presisi diberikan jika metode memberikan simpangan baku relatif (RSD) atau koefisien variasi (CV) 2% atau kurang [8]. Hasil pengukuran ditunjukkan dalam Tabel 5.

**Tabel 5.** Penentuan Keterulangan

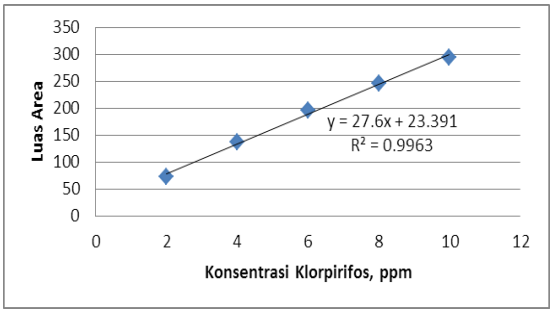
| Pengukuran ke- | Luas Area |
|---|---|
| 1 | 138,33823 |
| 2 | 139,04747 |
| 3 | 138,96838 |
| 4 | 138,69792 |
| 5 | 139,07574 |
| 6 | 139,10226 |
| 7 | 138,62209 |
| Rerata | 138,83601 |
| Simpangan Baku (SB) | 0,28960094 |
| Koefisien Variasi (KV) | 0,20859209% |

Berdasarkan hasil penenlitian seperti yang ditunjukkan dalam Tabel 5, dapat dilihat bahwa nilai koefisien variasi (KV) untuk penentuan klorpirifos 2 ppm adalah 0,2086%. Karena nilai KV lebih kecil dari 2% maka metode analisis tersebut mempunyai presisi yang baik [8]. Dengan demikian dapat disimpulkan bahwa sistem operasional alat dan analisis memiliki nilai keterulangan yang baik terhadap metode dengan respon yang relatif konstan, sehingga hasil pengukuran memiliki nilai presisi yang memenuhi persyaratan.

*b. Linearitas dan Kurva Kalibrasi*

Linearitas adalah kemampuan metode analisis memberikan respon proporsional terhadap konsentrasi analit dalam sampel [10]. Linearitas biasanya dinyatakan dalam istilah variansi sekitar arah garis regresi yang dihitung berdasarkan persamaan matematik data yang diperoleh dari hasil uji analit dalam sampel dengan berbagai konsentrasi analit [8]. Sebagai parameter adanya hubungan linier digunakan koefisien korelasi $r$ pada analisis regresi linier $y = a + bx$. Hubungan linier ideal dicapai jika nilai $a = 0$ dan $r = +1$ atau $-1$, bergantung pada arah garis [10].

Berdasarkan hasil pengkuran 5 seri larutan satndar klorpirifos dengan rentang konsentrasi 2 – 10 ppm, didapatkan kurva kalibrasi dengan persamaan garis regresi $y = 27{,}6x + 23{,}391$ dan nilai koefisien korelasi $R^2$ = 0,9963 seperti ditunjukkan dalam Gambar 3.




**Gambar 3.** Kurva kalibrasi Larutan Standar Klorpirifos

Nilai regresi yang baik adalah $R^2 > 0,99$ [4][8]. Dengan demikian, nilai koefisien korelasi yang diperoleh telah memenuhi persyaratan untuk digunakan dalam pengukuran analisis rutin.

*c. Batas Deteksi dan Batas Kuantitasi*

Batas deteksi (LOD) adalah jumlah terkecil analit dalam sampel yang dapat dideteksi yang masih memberikan respon signifikan dibandingkan dengan blangko, sedangkan batas kuantitasi (LOQ) merupakan parameter pada analisis renik dan diartikan sebagai kuantitas terkecil analit dalam sampel yang masih dapat memenuhi kriteria cermat dan seksama [8]. Nilai LOD dan LOQ dihitung secara statistik melalui garis regresi dari kurva kalibrasi.

Berdasarkan hasil penelitian yang dilakukan untuk penentuan klorpirifos dengan metode HPLC, diperoleh nilai LOD sebesar 0,67 ppm dan nilai LOQ sebesar 2,24 ppm. Batas deteksi berguna dalam memastikan suatu respon yang ditimbulkan suatu analisis [11].

*4. Penetapan Kadar Klorpirifos dalam Sampel*

Tujuan penelitian ini adalah untuk menentukan konsentrasi klorpirifos dalam sampel buah tomat, cabai rawit, dan wortel dengan menggunakan HPLC yang telah dioptimasi dan divalidasi sebelumnya [7]. Hasil pengukuran dan perhitungan kadar klorpirifos dalam sampel buah tomat, cabai rawit, dan wortel yang dianalisis dari beberapa pasar tradisional di Sulawesi Utara diringkaskan pada Tabel 6.

**Table 6.** Konsentrasi Klorpirifos dalam Tomat, Cabai Rawit, dan Wortel yang diambil dari Beberapa Pasar Tradisional di Sulawesi Utara

| No | Nama Sampel | Lokasi Sampling | Perlakuan | [Klorpirifos] (mg/kg) |
|---|---|---|---|---|
| 1 | Tomat | Pasar Bersehati Tomohon | Dicuci | 0.0314 |
| | | | Tidak Dicuci | 0.0471 |
| | | Pasar Karombasan Manado | Dicuci | 0.0146 |
| | | | Tidak Dicuci | 0.0594 |
| | | Pasar Kawangkoan | Dicuci | Tidak Terdeteksi |
| | | | Tidak Dicuci | 0.3150 |
| 2 | Cabai Rawit | Pasar Bersehati Tomohon | Dicuci | 0.1459 |
| | | | Tidak Dicuci | 0.1875 |
| | | Pasar Karombasan Manado | Dicuci | 0.1397 |
| | | | Tidak Dicuci | 0.1502 |
| | | Pasar Kawangkoan | Dicuci | Tidak Terdeteksi |
| | | | Tidak Dicuci | Tidak Terdeteksi |
| 3 | Wortel | Pasar Bersehati Tomohon | Dicuci | 0.0105 |
| | | | Tidak Dicuci | 0.0304 |
| | | Pasar Karombasan Manado | Dicuci | -0.0003 |
| | | | Tidak Dicuci | 0.0034 |
| | | Pasar Kawangkoan | Dicuci | 0.0040 |
| | | | Tidak Dicuci | 0.0049 |

Berdasarkan data pada Tabel 6, dapat dilihat bahwa pestisida dengan bahan aktif klorpirifos terdeteksi hampir pada semua sampel yang dianalisis, walaupun kadarnya masih berada di bawah nilai BMR yang ditetapkan, yaitu 1 mg/kg. kadar klorpirifos tertinggi dijumpai pada sampel buah tomat yang berasal dari pasar Kawangkoan Minahasa, yaitu sebesar 0,3150 mg/kg sampel. Hasil penelitian ini juga memperlihatkan bahwa sampel yang dicuci terlebih dahulu sebelum diekstraksi dapat menurunkan kadar pestisidanya. Hasil penelitian ini diharapkan dapat menjadi bahan pertimbangan bagi instansi terkait yang ada di Sulawesi Utara agar lebih sering memonitor dan mengevaluasi tentang keberadaan dan penggunaan pestisida.

**Ucapan Terimakasih**

Terima kasih disampaikan kepada Direktorat Riset dan Pengabdian kepada Masyarakat (DRPM) Kementrian Riset, Teknologi

dan Pendidikan Tinggi, yang telah mendanai penelitian ini melalui skema Penelitian Dosen Pemula (PDP).

**Kesimpulan**

Berdasarkan hasil yang telah dicapai dalam penelitian ini, maka dapat disimpulkan bahwa metode HPLC yang telah dioptimasi dan divalidasi dapat digunakan untuk menentukan kadar residu klorpirifos dalam sampel buah tomat, cabai rawit, dan wortel dengan ketelitian yang tinggi, sehingga dapat juga digunakan untuk analisis rutin senyawa klorpirifos dalam berbagai sampel.

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Eksakta Vol. 18 No. 2, Oktober 2017
http://eksakta.ppj.unp.ac.id
**E-ISSN : 2549-7464**
**P-ISSN : 1411-3724**

# ANALISIS RESIDU KLORPIRIFOS DALAM SAYURAN KUBIS DENGAN METODE HPLC DI BEBERAPA PASAR TRADISIONAL DI SULAWESI UTARA

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## ABSTRACT

One type of pesticide that is widely used is a group of organophosphates with chlorpyrifos as the active ingredients, because it has more favorable characteristics such as easy to decompose and short persistence time. However, the use of pesticides in addition to leaving residues that can cause environmental pollution, also cause disruption to human health and inhibit trade. Therefore, it is necessary to monitor the use of pesticides through the fulfillment of the maximum residual limit (MRL) so as to ensure food safety by limiting pesticide residue levels on agricultural products. The purpose of this study was to determine the residual levels of chlorpyrifos in cabbage vegetables with previously optimized and validated HPLC methods. The sample of this research is vegetable cabbage taken from several traditional markets in North Sulawesi. Based on the results of the study, it was found that chlorpyrifos were detected in all samples analyzed, although the levels were still below the specified MRL value. The results of this study can be a consideration for consumers and relevant agencies in North Sulawesi to more frequently monitor and evaluate the existence and use of these compounds.

**Keywords:** *chlorpyrifos, HPLC, cabbage*

## PENDAHULUAN

Penggunaan pestisida di Indonesia cukup tinggi mengingat Indonesia adalah negara agraris yang mengandalkan sektor pertanian. Berdasarkan data Departemen Petanian tahun 2011, terjadi peningkatan penggunaan pestisida di Indonesia, yaitu pada tahun 2006 tercatat sebanyak 1.557 formulasi pestisida yang terdaftar meningkat menjadi 2.628 pada tahun 2010 (Departemen Pertanian, 2011). Padahal penggunaan pestisida selain dapat meninggalkan residu yang dapat menyebabkan pencemaran lingkungan, juga menyebabkan gangguan pada kesehatan manusia dan menghambat perdagangan (Chen dkk., 2011; Departemen Pertanian, 2011). Oleh karena itu, perlu dilakukan pengawasan terhadap penggunaan pestisida melalui pemenuhan nilai batas maksimum residu (BMR) sehingga dapat menjamin keamanan pangan dengan caramembatasi kadar residu pestisida pada komoditas hasil-hasil pertanian.

Salah satu jenis pestisida yang banyak digunakan petani adalah pestisida golongan organofosfat dengan bahan aktif klorpirifos. Hal ini disebabkan karena pestisida golongan organofosfat memiliki karakteristik yang lebih menguntungkan

E-ISSN : 2549-7464, P-ISSN : 1411-3724

dibandingkan dengan pestisida organoklorin, seperti mudah terurai dan waktu persistennya yang singkat (Chen dkk., 2011). Pestisida organofosfat melindungi tanaman dari hama dengan menghambat aktivitas enzim asetilkolineterase pada serangga. Penggunaannya dengan cara disemprot pada tanaman atau tanah, menyebabkan residunya dapat ditemukan di permukaan air tanah, buah-buahan, sayuran dan air minum (Yao dkk., 2001).

Keberadaan pestisida organofosfat dengan bahan aktif klorpirifos dalam produk-produk pertanian telah dilaporkan oleh beberapa peneliti di Indonesia.Panggabean (2016) dalam penelitiannya tentang analisis residu klorpirifos dalam sayur-sayuran dengan teknik HPLC, menemukan adanya residu klorpirifos dalam sampel sayur kubis yang dianalisis dengan kadar 0,131 ± 0,008 µg/kg. Kadar tersebut masih jauh di bawah nilai BMR yang ditetapkan untuk klorpirifos dalam sayur kubis, yaitu 1 mg/kg (SNI 7313:2008).Marzuki dkk.,(2014) juga menemukan adanya residu klorpirifos pada sampel uji Sawi Hijau di Kabupaten Gowa, walaupun masih dalam ambang toleransi BMR. Berdasarkan hasil penelitian Triani dkk., (2013), rata-rata residu pestisida klorpirifos pada kacang panjang di Kecamatan Baturiti Kecamatan Marga dan Kecamatan Kerambitan masing-masing sebesar 0,0397 mg/kg, 0,0169 mg/kg, dan 0,0118 mg/kg, hasil tersebut masih di bawah BMR, sedangkan dikecamatan Penebel sebesar 0,2447 mg/kg masih berada di atas BMR. Namun sejauh ini, berdasarkan penelusuran literatur maupun pengamatan di lapangan, masih sangat kurang penelitianyang dilakukan untuk analisis residu pestisida pada hasil-hasil pertanian di daerah Sulawesi Utara.

Oleh karena itu, pada penelitian ini telah dilakukan penentuan kadarresidu klorpirifos dalam sayur kubis yang diambil dari beberapa pasar tradisional yang ada di Sulwesi Utara menggunakan metode HPLC yang telah dioptimasi dan divalidasi sebelumnya.

## METODE PENELITIAN

### Alat

Peralatan yang digunakan dalam penelitian ini antara lain: seperangkat alat HPLC (Agilent 1260 Infinity Binary LC) yang dilengkapi dengan detektor DAD, utosampler, dan kolom Zorbax Eclipse Plus C18 (3,5 µm; 2,1 x 100 mm), Spektrofotometer UV-Vis PerkinElmer Lambda 25, rotavapor, sonikator, neraca analitik, penyaring vakum beserta saringan berpori 0,4 - 0,45 µm, blender, corong pisah dengan tutup, penangas air, pompa vakum, dan alat gelas yang lazim.

### Bahan

Bahan-bahan yang digunakan antara lain sampel daun kubis (*Brassica oleracea*) yang diambil dari Pasar Bersehati Tomohon, Pasar Karombasan Manado, Pasar Tondano, Pasar Langoan, dan Pasar Kawangkoan, Klorpirifos (Kemurnian ≥ 99,9%, Merck), Asetonitril (gradient grade, ≥ 99,9%, Merck), etilasetat, $Na_2SO_4$ anhidrat, aquabidest.

### Prosedur Penelitian

E-ISSN : 2549-7464, P-ISSN : 1411-3724

Penelitian ini terdiri dari beberapa tahap, yakni:

***1. Penentuan panjang gelombang serapan maksimum klorpirifos***

Panjang gelombang serapan maksimum klorpirifos ditentukan dengan mengukur serapan larutan baku klorpirifos 10 ppm menggunakan spektrofotometer UV/Vis Lambda 25.

***2. Penentuan kondisi optimum HPLC***

Kondisi optimum HPLC ditentukan menggunakan kolom Zorbax Eclipse Plus C18 (3,5 µm; 2,1 x 100 mm) menggunakan detektor DAD pada panjang gelombang otptimum yang telah diperoleh sebelumnya. Penentuan kondisi optimum HPLC meliputi:

*a. PenentuanKomposisi Fasa Gerak*

Fasa gerak yang digunakan dalam penelitian ini adalah air dan asetonitril dengan komposisi air : asetonitril divariasikan dari 100:0 sampai 0 : 100. Sebanyak 5 µL larutan baku klorpirifos 2 ppm diinjeksikan ke dalam kolom HPLC kemudian ditentukan waktu retensi dan luas area puncak kromatogram masing-masing komposisi fasa gerak tersebut.

*b. Penentuan Laju Alir Fasa Gerak*

Laju alir fasa gerak dipalajari dengan mengukur waktu retensi dan luas area puncak kromatogram larutan baku klorpirifos 2 ppm pada laju alir yang divariasikan dari 0,3 – 0,9 mL/menit menggunakan komposisi fasa gerak optimum yang telah ditentukan sebelumnya.

*c. Penentuan Volume Injeksi*

Volume injeksi sampel dipelajari dengan mengukur luas area puncak kromatogram larutan baku klorpirifos 2 ppm dengan volume injeksi divariasikan dari 5 – 30 µL menggunakan komposisi dan laju alir fasa gerak optimum yang telah ditentukan sebelumnya.

***3. Penentuan Kinerja Analitik***

Setelah ditentukan kondisi optimum HPLC, selanjutnya ditentukan kinerja analitik yang meliputi:

*a. Presisi*

Presisi ditetapkan berdasarkan keterulangan (*repeatability*) yang ditentukan dengan mengukur luas area puncak kromatogram larutan baku klorpirifos 2 ppm pada kondisi optimum HPLC. Pengukuran diulangi sebanyak 7 kali kemudian ditentukan simpangan baku relatif (SBR) atau keoefisien variasi (KV).

*b. Linearitas dan Kurva kalibrasi*

Kurva kalibrasi dibuat dengan mengukur luas area puncak kromatogram larutan baku klorpirifos dengan konsentrasi yang divariasikan dari 0 – 10 ppm menggunakan kondisi optimum HPLC yang telah ditentukan sebelumnya. Kemudian dibuat kurva kalibrasi dengan mengalurkan luas area puncak kromatogram (sebagai sumbu-y) terhadap konsentrasi larutan baku klorpirifos (sebagai sumbu-x), lalu dihitung persamaan garis regresi dan koefisien korelasinya.

*c. Batas Deteksi dan Batas Kuntitasi*

Dalam penelitian ini, nilai batas deteksi (LOD) dan batas kuantitasi (LOQ) dihitung secara statistik melalui

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garis regresi dari kurva kalibrasi (Harmita, 2004).Nilai LOD dihitung menggunakan persamaan:

$$LOD = \frac{3S_{(y/x)}}{b} \quad (1)$$

Sedangkan nilai LOQ dihitung menggunakan persamaan:

$$LOQ = \frac{10\,S_{(y/x)}}{b} \quad (2)$$

Dimana $S_{(y/x)}$ adalah simpangan baku residual, dan *b* adalah slope dari persamaan garis regresi linier.

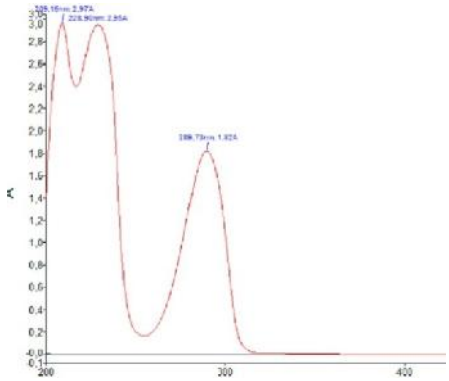
### *4. Preparasi Sampel dan Penetapan Kadar Residu Klorpirifos*

Sampel daun kubis dibagi dalam 2 kelompok berdasarkan cara perlakuan yang berbeda, yaitu sampel daun kubis yang dicuci dengan air sebelum diekstraksi dan sampel daun kubis tanpa dicuci dengan air.Sejumlah 300 gram sampel daun kubis dari hasil perlakuan di atas diblender sampai homogen, kemudian ditimbang sebanyak 25 gram dan ditambahkan 25 gram natrium sulfat anhidrat dan 50 mL etilasetat, kemudian diekstraksi selama 10 menit dengan alat ekstraksi khusus. Ekstraknya kemudian disaring dengan penyaring vakum dan filtratnya ditampung, kemudian ampasnya diekstraksi kembali dengan 50 mL etil asetat sebanyak tiga kali.Filtrat hasil ekstraksi pertama sampai ketiga dicampurkan kemudian dipekatkan dengan rotavapor pada suhu 35$^{o}$C hingga menghasilkan ekstrak pekat sebanyak 1 – 3 mL (Komisi Pestisida, 2004).Selanjutnya sampel diencerkan dengan eluen dan diukur dengan HPLC kemudian ditentukan kadar klorpirifos.

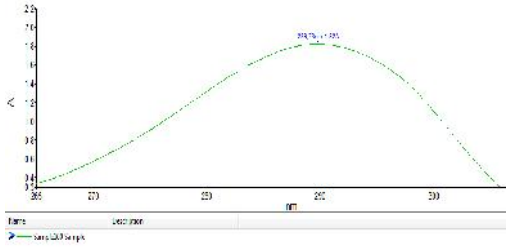
## HASIL DAN PEMBAHASAN

### 1. Panjang Gelombang Serapan Maksimum Klorpirifos

Panjang gelombang serapan maksimum klorpirifos ditentukan dengan mengukur serapan larutan baku klorpirifos 10 mg/L menggunakan spektrofotometer UV/Vis Lambda 25 pada rentang panjang gelombang 190 – 700 nm. Berdasarkan hasil pengukuran diperoleh tiga daerah serapan larutan baku klorpirifos, yaitu pada λ = 209 nm, λ = 229 nm, dan λ = 289 nm, seperti diperlihatkan dalam Gambar 1a. Panjang gelombang serapan maksimum klorpirifos yang dipilih untuk digunakan dalam penelitian ini adalah λ = 289 nm, karena pada panjang gelombang tersebut tidak terjadi tumpang tindih, seperti diperlihatkan dalam Gambar 1b.



(a)



(b)

Gambar 1. (a) Panjang gelombang serapan klorpirifos, (b) Serapan klorpirifos pada λ = 289 nm yang diperbesar

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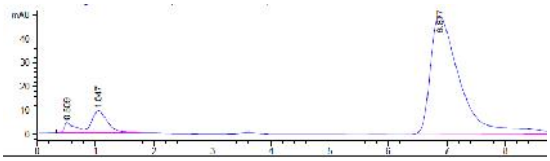
Panjang gelombang serapan maksimum klorpirifos tersebut selanjutnya digunakan untuk melakukan optimasi kondisi pengukuran HPLC menggunakan detektor DAD pada panjang gelombang tersebut.

## 2. Penentuan Kondisi Optimum HPLC

*a. Pengaruh Komposisi Fasa gerak*

Fasa gerak yang digunakan dalam penelitian ini adalah air dan asetonitril yang bersifat lebih polar dibandingkan fasa diam, yakni C18 yang bersifat nonpolar.Komposisi fasa gerak optimum ditentukan berdasarkan luas area puncak kromatogram dan waktu retensi, karena luas puncak merupakan parameter yang lebih akurat untuk pengukuran kuantitatif (Panggabean dkk, 2009).

Berdasarkan hasil pengukuran didapati bahwa kromatogram klorpirifos mulai terbaca pada komposisi air : asetonitril 50 : 50 seperti ditunjukkan dalam Gambar 2.



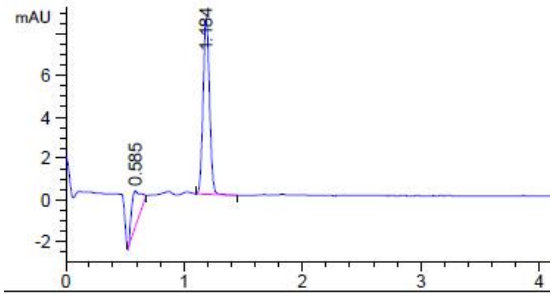
Gambar 2. Kromatogram larutan baku klorpirifos 2 ppm pada komposisi air : asetonitril (50:50)

Hasil pengukuran selengkapnya pada berbagai komposisi fasa gerak air:asetonitril diperlihatkan dalam Tabel 1.

Tabel 1. Pengaruh komposisi air:asetonitril terhadap waktu retensi dan luas area

| Komposisi Air:Asetonitril | Waktu Retensi (menit) | Luas Area(mAU*s) |
|---|---|---|
| 50 : 50 | 6,877 | 1753,36926 |
| 40 : 60 | 6,724 | 32,82681 |
| 30 : 70 | 3,055 | 32,73163 |
| 20 : 80 | 1,797 | 32,44448 |
| 10 : 90 | 1,184 | 32,23305 |
| 0 : 100 | 0,999 | 32,98140 |

Berdasarkan data pada Tabel 1 dapat dilihat bahwa jika komposisi astonitril diperbesar, maka luas area puncak kromatogram klorpirifos semakin berkurang namun waktu retensinya semakin singkat.Luas area puncak kromatogram klorpirifos terbesar terbaca pada komposisi air:asetonitril (50:50), namun waktu retensinya lebih lama dan kromatogram yang dihasilkan tidak simetris. Oleh karena itu, komposisi fasa gerak air : asetonitril yang digunakan dalam penelitian ini adalah 10 : 90, karena waktu pengukurannya lebih singkat dan luas area puncak kromatogramnya masih cukup baik (Gambar 3).




Gambar 3. Kromatogram lorpirifos pada komposisi fasa gerak air : asetonitril (10 : 90)

*b. Pengaruh Laju Alir Fasa Gerak*

Pengukuran laju alir fasa gerak dilakukan menggunakan komposisi fasa gerak optimum yang telah ditentukan sebelumnya, yaitu pada perbandingan air : asetonitril 10 : 90. Hasil pengkuran tersebut ditunjukkan dalam Tabel 2 berikut.

Tabel 2. Pengaruh Laju Alir Fasa Gerak terhadap Waktu Retensi dan Luas Area

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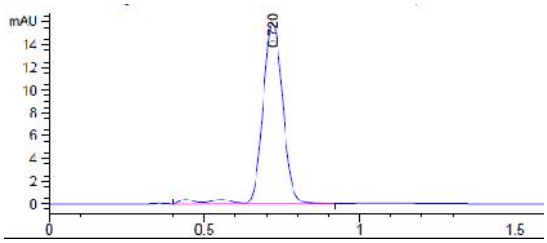
| Laju Alir Fasa Gerak (mL/menit) | Waktu Retensi (menit) | Luas Area(mAU*s) |
|---|---|---|
| 0,30 | 1,682 | 162,69608 |
| 0,50 | 1,006 | 97,39092 |
| 0,70 | 0,720 | 72,81155 |
| 0,90 | 0,561 | 61,86494 |

Berdasarkan data pada Tabel 2, dapat dilihat bahwa semakin tinggi laju alir fasa gerak, semakin singkat waktu retensi dan semakin kecil luas area puncak kromatogram yang dihasilkan. Kromatogram terbaik dengan waktu retensi lebih singkat diperoleh pada laju alir 0,70 mL/menit (Gambar 4). Pada laju alir 0,90 mL/menit, kromatogram yang dihasilkan tidak simetris lagi.




Gambar 4. Kromatogram klorpirifos pada laju alir fasa gerak 0,70 mL/menit

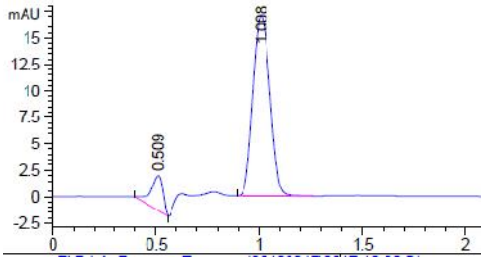
*c. Pengaruh Volume Injeksi Sampel*

Pengukuran parameter volume injeksi sampel dilakukan menggunakan komposisi dan laju alir fasa gerak optimum yang telah ditentukan sebelumnya, yaitu komposisi air : asetonitril 10:90 dan laju alir 0,70 mL/menit. Hasil pengkuran tersebut ditunjukkan dalam Tabel 3 berikut.

Tabel 3. Pengaruh Volume Injeksi Sampel terhadap Waktu Retensi dan Luas Area

| Volume Injeksi Sampel (µL) | Waktu Retensi (menit) | Luas Area(mAU*s) |
|---|---|---|
| 5,0 | 1,184 | 32,23305 |
| 10,0 | 1,008 | 65,02857 |
| 15,0 | 1,008 | 97,40105 |
| 20,0 | 1,004 | 129,75168 |
| 25,0 | 1,001 | 178,27931 |
| 30,0 | 0,993 | 210,57770 |

Berdasarkan data pada Tabel 3 dapat dilihat bahwa luas area puncak kromatogram larutan standar klorpirifos semakin besar jika volume injeksi sampel diperbesar, namun pada volume injeksi ≥ 20 µL, kromatogram larutan baku klorpirifos menjadi tidak simetri lagi. Oleh karena itu, dalam penelitian ini digunakan volume injeksi sampel 15 µL, karena menghasilkan kromatogram yang lebih baik seperti diperlihatkan dalam Gambar 5.




Gambar 5. Kromatogram klorpirifos pada volume injeksi sampel 15 µL

Berdasarkan hasil otimasi parameter-parameter kromatografik di atas, maka dapat diringkaskan kondisi optimum HPLC untuk penetapan residu klorpirifos seperti ditunjukkan dalam Tabel 4 berikut.

Tabel 4. Kondisi optimum HPLC untuk penentuan klorpirifos

| Parameter | Hasil Pengukuran |
|---|---|
| Kolom | Zorbax Eclipse Plus C18 (3,5 µm; 2,1 x 100 mm) |
| Detektor | DAD |
| Panjang gelombang | 289 nm |

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| | |
|---|---|
| Komposisi Fasa Gerak | Air : Asetonitril (10:90) |
| Laju Alir Fasa Gerak | 0,70 mL/menit |
| Volume Injeksi | 15 µL |

**3. Penentuan Kinerja Analitik**

*a. Presisi*

Presisi merupakan ukuran yang menunjukkan derajat kesesuaian antara hasil uji individual, diukur melalui penyebaran hasil individual dari rata-rata jika prosedur diterapkan secara berulang pada sampel-sampel yang diambil dari campuran yang homogen [6].Pada penelitian ini, presisi ditetapkan berdasarkan keterulangan luas area kromatogram hasil analisa dengan 7 kali pengulangan pada larutan standar. Kriteria presisi diberikan jika metode memberikan simpangan baku relatif (RSD) atau koefisien variasi (CV) 2% atau kurang (Harmita, 2004). Hasil pengukuran ditunjukkan dalam Tabel 4.

Tabel 5. Penentuan Keterulangan

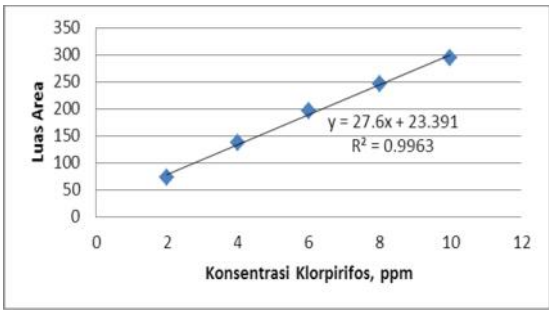
| Pengukuran ke- | Luas Area |
|---|---|
| 1 | 138,33823 |
| 2 | 139,04747 |
| 3 | 138,96838 |
| 4 | 138,69792 |
| 5 | 139,07574 |
| 6 | 139,10226 |
| 7 | 138,62209 |
| Rerata | 138,83601 |
| Simpangan Baku (SB) | 0,28960094 |
| Koefisien Variasi (KV) | 0,20859209% |

Berdasarkan hasil penenlitian seperti yang ditunjukkan dalam Tabel 5, dapat dilihat bahwa nilai koefisien variasi (KV) untuk penentuan klorpirifos 2 ppm adalah 0,2086%. Karena nilai KV lebih kecil dari 2% maka metode analisis tersebut mempunyai presisi yang baik [6].Dengan demikian dapat disimpulkan bahwa sistem operasional alat dan analisis memiliki nilai keterulangan yang baik terhadap metode dengan respon yang relatif konstan, sehingga hasil pengukuran memiliki nilai presisi yang memenuhi persyaratan.

*b. Linearitas dan Kurva Kalibrasi*

Linearitas adalah kemampuan metode analisis memberikan respon proporsional terhadap konsentrasi analit dalam sampel (Riyanto, 2014).Linearitas biasanya dinyatakan dalam istilah variansi sekitar arah garis regresi yang dihitung berdasarkan persamaan matematik data yang diperoleh dari hasil uji analit dalam sampel dengan berbagai konsentrasi analit (Harmita, 2004).Sebagai parameter adanya hubungan linier digunakan koefisien korelasi $r$ pada analisis regresi linier $y = a + bx$. Hubungan linier ideal dicapai jika nilai $a = 0$ dan $r = +1$atau $-1$, bergantung pada arah garis (Riyanto, 2014).

Berdasarkan hasil pengkuran 5 seri larutan satndar klorpirifos dengan rentang konsentrasi 2 – 10 ppm, didapatkan kurva kalibrasi dengan persamaan garis regresi $y = 27{,}6x + 23{,}391$ dan nilai koefisien korelasi $R^2$ = 0,9963 seperti ditunjukkan dalam Gambar 3.




Gambar 3. Kurva kalibrasi Larutan Standar Klorpirifos

Nilai regresi yang baik adalah $R^2$> 0,99 (Miller dan Miller, 1991). Dengan demikian,

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nilai koefisien korelasi yang diperoleh telah memenuhi persyaratan untuk digunakan dalam pengukuran analisis rutin.

*c. Batas Deteksi dan Batas Kuantitasi*

Batas deteksi (LOD) adalah jumlah terkecil analit dalam sampel yang dapat dideteksi yang masih memberikan respon signifikan dibandingkan dengan blangko, sedangkan batas kuantitasi (LOQ) merupakan parameter pada analisis renik dan diartikan sebagai kuantitas terkecil analit dalam sampel yang masih dapat memenuhi kriteria cermat dan seksama (Harmita, 2004).Nilai LOD dan LOQ dihitung secara statistik melalui garis regresi dari kurva kalibrasi.

Berdasarkan hasil penelitian yang dilakukan untuk penentuan klorpirifos dengan metode HPLC, diperoleh nilai LOD sebesar 0,67 ppm dan nilai LOQ sebesar 2,24 ppm. Batas deteksi berguna dalam memastikan suatu respon yang ditimbulkan suatu analisis (Panggabean dkk., 2014).

**4. Penetapan Kadar Klorpirifos dalam Sampel**

Tujuan penelitian ini adalah untuk menentukan konsentrasi klorpirifos dalam sayur kubis dengan menggunakan HPLC yang telah dioptimasi dan divalidasi sebelumnya. Hasil pengukuran dan perhitungan kadar klorpirifos dalam sampel sayur kubis yang dianalisis dari beberapa pasar tradisional di Sulawesi Utara dapat dilihat pada Tabel 6.

Table 6. Konsentrasi Klorpirifos dalam Sayur Kubis yang diambil dari Beberapa Pasar Tradisional di Sulawesi Utara

| No. | Lokasi Sampel | Perlakuan | [Klorpirifos] (mg/kg) |
|---|---|---|---|
| 1 | Pasar Bersehati Tomohon | Dicuci | 0.2526 |
| | | Tidak Dicuci | 0.2912 |
| 2 | Pasar Karombasan Manado | Dicuci | 0.1969 |
| | | Tidak Dicuci | 0.2157 |
| 3 | Pasar Kawangkoan | Dicuci | 0.4961 |
| | | Tidak Dicuci | 0.5870 |
| 4 | Pasar Langowan | Dicuci | 0.3441 |
| | | Tidak Dicuci | 0.5878 |
| 5 | Pasar Tondano | Dicuci | 0.0472 |
| | | Tidak Dicuci | 0.0533 |

Berdasarkan data pada Tabel 6, dapat dilihat bahwa klorpirifos terdeteksi pada semua sampel yang dianalisis, walaupun kadarnya masih berada di bawah nilai BMR yang ditetapkan, yaitu 1 mg/kg.Hasil penelitian ini dapat menjadi bahan pertimbangan bagi para konsumen dan instansi terkait yang ada di Sulawesi Utara agar lebih sering memonitor dan mengevaluasi tentang keberadaan dan penggunaan senyawa ini.

## KESIMPULAN

Berdasarkan hasil yang telah dicapai dalam penelitian ini, maka dapat disimpulkan bahwametode HPLC yang telah dioptimasi dan divalidasi dapat digunakan untuk menentukan kadarresidu klorpirifos dalam sayur kubis dengan ketelitian yang tinggi, sehinggadapat juga digunakan untuk analisisrutin senyawa klorpirifos dalam berbagai sampel.

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# ANALISIS RESIDU KLORPIRIFOS DALAM SAYUR-SAYURAN DENGAN TEKNIK *HIGH PERFORMANCE LIQUID CHROMATOGRAPHY* (HPLC)

# ANALYSIS OF CHLORPYRIFOS RESIDUE IN VEGETABLES BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) TECHNIQUE

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## ABSTRACT

The research about analysis of chlorpyrifos residue in vegetables by using High Performance Liquid Chromatography (HPLC) technique has been done. To obtain the optimal measurement results, the measurement performed several important parameters in the chromatographic system was composition of mobile phase, volume injection sample, flow rate and pH eluent. Optimum measurement conditions obtained was mobile phase composition (water : methanol) with 70 : 30, volume injection sample are 5 μL, flow rate are 0.5mL/menit and pH eluent are 7. The analytical performance that obtained is good showed with the reproducibility value as percentage coefficient variance (% CV) was 0.0664%, limit of detection (LOD) was 0.44 ppm, with a recovery percentage of > 95%. The results obtained showed the HPLC technique can be used for the routine analysis in the determination of chlorpyrifos for the vegetable samples.

**Keywords:** *Chlorpyrifos, Vegetables, HPLC.*

## PENDAHULUAN

Pertambahan jumlah penduduk pada beberapa dekade belakangan ini sangat besar. Hal ini tentunya berdampak dalam penyediaan kebutuhan bahan makanan yang meningkat dari waktu ke waktu. Upaya produksi pangan sering menghadapi kendala serangan hama yang menyebabkan gagal panen atau minimal hasil panen berkurang. Salah satu cara yang terbukti meningkatkan produksi hasil tanaman pangan adalah penggunaan pestisida, namum di sisi lain karena pestisida adalah bahan kimia beracun, pemakaian pestisida berlebihan dapat menjadi sumber pencemar bagi bahan pangan, air dan lingkungan hidup. Residu sejumlah bahan kimia yang ditinggalkan melalui berbagai siklus, langsung atau tidak langsung, dapat sampai ke manusia, terhirup melalui pernafasan, dan masuk ke saluran pencernaan bersama makanan dan air minum [1].

Dewasa ini jenis pestisida yang paling banyak beredar dan digunakan dalam pengendalian hama penyakit tanaman secara terpadu adalah jenis organofosfat dan karbamat. Senyawa organofosfat dan karbamat bersifat menghambat enzim *cholinesterase,* yaitu enzim yang berperan dalam penerusan rangsangan syaraf. Peracunan dapat terjadi karena gangguan dalam fungsi susunan syaraf yang akan menyebabkan kematian atau dapat pulih kembali. Umur residu dari organofosfat dan karbamat ini tidak berlangsung lama sehingga peracunan kronis terhadap lingkungan cenderung tidak terjadi karena faktor-faktor lingkungan mudah menguraikannya menjadi komponen yang tidak beracun [2][3].

Klorpirifos (*o,o-dietil-o*-3,5,6-*trichloropyridin*-2-*il-phosphorothioate*) adalah kristal organofosfat insektisida yang menghambat *acetylcholinesterase* dan digunakan untuk mengontrol hama serangga. Klorpirifos ini cukup beracun dan paparan kronis dapat menyebabkan efek neurologis, gangguan pertumbuhan, dan gangguan autoimun. Klorpirifos adalah salah satu dari sekitar 100 insektisida organofosfat yang tersebar luas di pasaran saat ini dan digunakan untuk membunuh hama serangga dengan mengganggu sistem saraf mereka. Klorpirifos memiliki keuntungan lebih dari produk lainnya yaitu efektif terhadap berbagai tanaman pemakan serangga hama [4].

Jumlah Klorpirifos dalam bahan makanan terutama dalam sayur-sayuran harus diketahui, karena senyawa ini sangat berbahaya bagi

kesehatan manusia yang mengkomsumsinya. Untuk itu diperlukan suatu metode analisis yang sensitif dan selektif yang dapat mendeteksi senyawa klorpirifos dan dapat digunakan secara rutin dan berbiaya murah. Salah satu teknik instrumentasi yang sensitif dan selektif untuk analisis senyawa organik dalam level renik adalah *High Performance Liquid hromatography* (HPLC) [5][6].

Pada penelitian ini telah dilakukan beberapa optimasi prosedur pengukuran untuk menentukan residu klorpirifos dalam sampel sayur-sayuran yang meliputi pengaruh komposisi eluen, volume injeksi, laju alir eluen, pH eluen dan penentuan kinerja analitik yang meliputi kebolehulangan, linearitas, batas deteksi dan pengaruh matriks, yang kemudian dapat diaplikasikan untuk menentukan kadar residu klorpirifos dalam sampel sayur-sayuran.

## METODOLOGI PENELITIAN

### Alat

Peralatan yang digunakan dalam penelitian ini adalah seperangkat instrument *High Performance Liquid Chromatography* (HPLC) Agilen 1100 Series kolom Shim-Pack VP-ODS (i.d. 4,6 x 250 mm) dan detektor UV, pompa vakum, pH-meter, labu ukur, pipet volume, beaker glass dan kertas saring whatman No.42.

### Bahan

Bahan-bahan yang digunakan dalam penelitian ini adalah larutan standar insektisida klorpirifos, buffer fosfat, metanol dan aquabides, asam asetat, $NH_4OH$ 1M, diklorometan, sayur-sayuran yaitu kembang kol, sawi putih dan kubis.

### Prosedur Penelitian

### Persiapan Alat HPLC

Kolom yang digunakan adalah Shim-Pack VP-ODS (4,6 x 250 mm). HPLC menggunakan detektor UV-Visible pada panjang gelombang 230 nm dengan sensitifitas 1,000 AUFS. Pompa menggunakan mode aliran tetap dengan sistem elusi gradien. Setelah alat HPLC dihidupkan, maka pompa dijalankan dengan fase gerak dibiarkan mengalir selama ± 30 menit sampai diperoleh *base line* yang menandakan sistem telah stabil.

### Optimasi Parameter Pengukuran HPLC

***Penentuan Fasa Gerak Air:Metanol***

Sebanyak 5 µl larutan standar diinjeksikan ke dalam kolom. Fasa gerak yang digunakan adalah metanol : air dengan komposisi perbandingan 0:100, 10:90, 20:80, 30:70, 40:60, 50:50. Ditentukan waktu retensi dan luas areanya.

***Penentuan Volume injeksi***

Diinjeksikan larutan standar dengan variasi volume yaitu 5- 20 µl pada komposisi fasa gerak yang optimum. Ditentukan waktu retensi dan luas areanya.

***Penentuan Laju Alir Fasa Gerak***

Larutan standar diinjeksikan ke dalam injektor HPLC menggunakan komposisi fasa gerak metanol : air dan volume injeksi optimum, dengan memvariasikan laju alir 0,3 - 1 mL/menit. Ditentukan waktu retensi dan luas areanya.

***Penentuan pH Fasa Gerak***

Sebanyak 5 µl larutan standar diinjeksikan ke dalam injektor pada komposisi fasa gerak optimum yang diatur pada berbagai variasi pH yaitu 3-8, pada volume sampel dan laju alir optimum yang diperoleh pada penelitian sebelumnya. Ditentukan waktu retensi dan luas areanya.

### Penentuan Kinerja Analitik

***Kebolehulangan***

Sebanyak 5 µL larutan standar 5 ppm diinjeksikan ke dalam kolom menggunakan fasa gerak dan laju alir yang optimum, diulangi sebanyak 7 kali, kemudian dicatat luas puncaknya dan dihitung faktor kapasitasnya.

***Linearitas dan Kurva Kalibrasi***

Kurva kalibrasi dibuat dengan memvariasikan konsentrasi larutan standar 0 - 25 ppm, kemudian masing-masing larutan standar diinjeksikan sebanyak 5 µl ke dalam kolom pada kondisi optimum. Pendeteksian menggunakan detektor UV pada panjang gelombang 230 nm. Direkam kromatogram dan dibuat kurva kalibrasi konsentrasi larutan standar-vs-luas puncak, lalu dihitung persamaan regresi dan koefisien korelasinya.

***Limit Deteksi***

Dalam penelitian ini LOD ditentukan dengan mengukur harga luas area dari konsentrasi klorpirifos terkecil yang masih dapat ditentukan dan dibedakan dari luas area yang diberikan oleh blanko dengan beberapa kali pengukuran. Limit deteksi dinyatakan sebagai perbandingan luas area standar (S) terhadap luas area blanko (N) atau S/N=3 [7][8].

***Pengaruh Matriks (% Recovery)***

Uji perolehan kembali dilakukan dengan metode spike. Pada tahap ini dipersiapkan larutan uji klorpirifos 5 ppm dan diencerkan dengan larutan sampel sayur sampai garis tanda. Larutan uji tersebut selanjutnya diinjeksikan ke injektor HPLC dan dihitung persentase perolehan kembalinya.

**Penentuan Konsentrasi Klorpirifos dalam Sampel**

***Preparasi Sampel***

Sayur kembang kol, sawi putih dan kubis dikeringkan, lalu dihaluskan dengan blender. Ditimbang sebanyak 20 gram dan tambahkan diklorometan sebanyak 100 mL, dimaserasi selama 48 jam. Disaring, lalu dimasukkan ke dalam corong pisah. Diekstraksi selama 60 menit, fraksi diklorometan diambil dan kemudian dimasukkan ke dalam gelas vial [5].

***Penetapan Kadar Residu Klorpirifos dalam Sampel***

Sampel diambil 25 mL dan dimasukkan ke dalam labu ukur 50 mL ditambahkan dengan eluen sampai tanda tera. Di kocok dan di saring, kemudian diinjeksikan ke dalam sistem HPLC melalui injektor. Direkam kromatogram dan di catat luas puncak. Kadarnya dihitung dengan mensubstitusikan luas puncak ke dalam persamaan regresi yang diperoleh dari kurva kalibrasi.

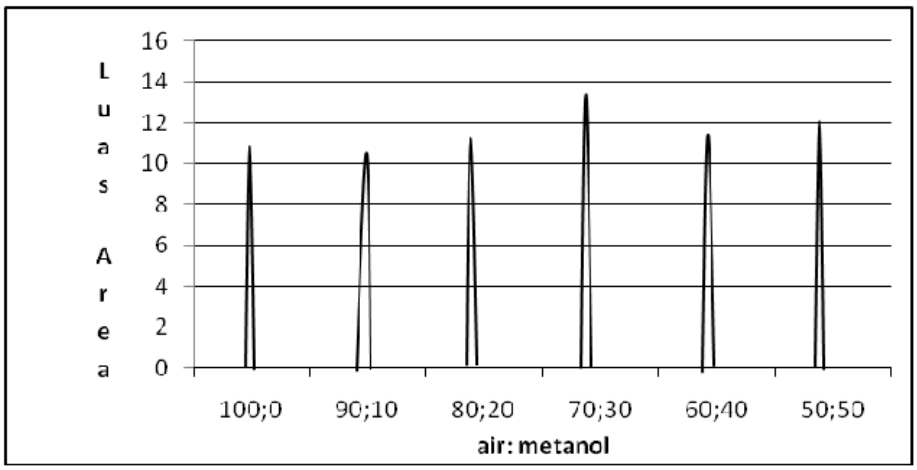
**HASIL DAN PEMBAHASAN**

Untuk mendapatkan kondisi pengukuran yang optimum HPLC yang digunakan dalam penentuan klorpirifos dalam sayur-sayuran, maka dilakukan pengukuran beberapa optimasi parameter kromatografik kemudian dilanjutkan dengan penentuan kinerja analitiknya.

**Optimasi Parameter Pengukuran HPLC**

**Pengaruh Komposisi Fasa Gerak Metanol : Air**

Penelitian ini menggunakan kromatografi cairan fasa terbalik (*reversed phase chromatography*, RPC), yaitu fasa gerak yang digunakan lebih polar bila dibandingkan dengan fasa diam yang bersifat nonpolar [9]. Penentuan hasil optimasi berdasarkan luas puncak kromatogram, karena luas puncak merupakan parameter yang lebih akurat untuk pengukuran kuantitatif [6]. Hasil pengukuran pengaruh komposisi fasa gerak dapat dilihat pada Gambar 1.



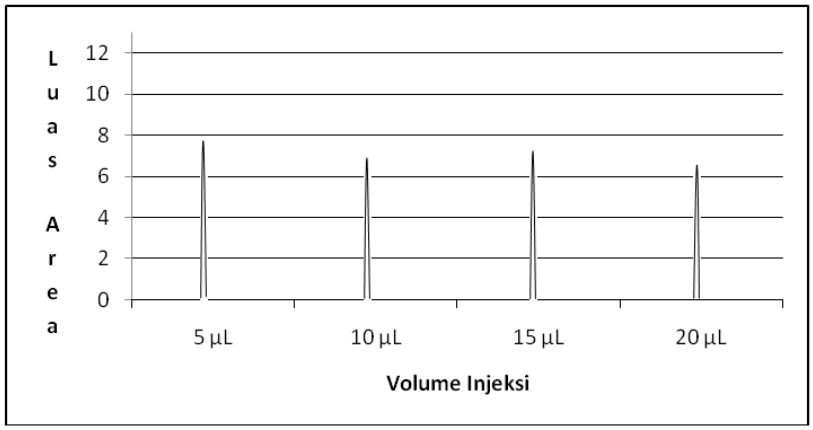

Gambar 1. Pengaruh komposisi fasa gerak air : metanol

Berdasarkan Gambar 1. pengukuran luas area paling besar didapatkan pada komposisi fasa gerak (air:metanol) 70:30, dibandingkan perbandingan komposisi yang lainnya. Pada penambahan metanol akan menurunkan kepolaran fasa gerak sehingga proses elusi terjadi lebih cepat, oleh karena itu waktu retensi menjadi singkat [6][10]. Maka, dapat disimpulkan bahwa pada komposisi fasa gerak air:metanol dengan perbandingan 70:30 adalah kondisi yang paling optimum untuk analisa klorpirifos.

**Pengaruh Volume Injeksi Sampel**

Dalam pengukuran parameter ini digunakan komposisi fasa gerak yang optimum hasil pengukuran sebelumnya, yaitu air:metanol dengan perbandingan 70:30. Hasil pengukuran dapat dilihat pada Gambar 2.

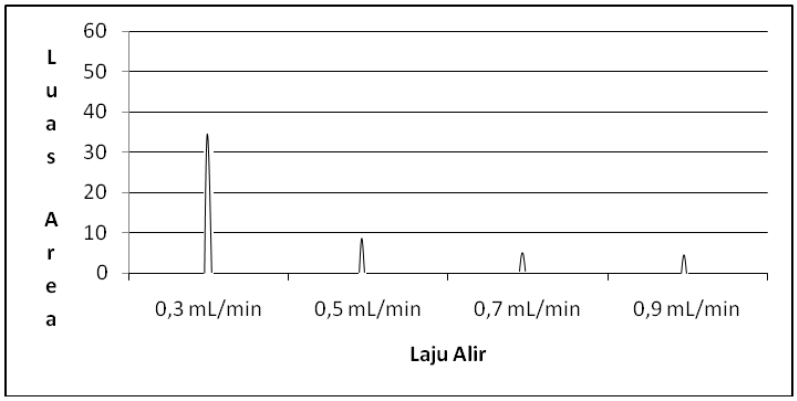
Pengukuran dengan nilai luas area paling tinggi diperoleh pada volume injeksi sampel 5 µL. Hal ini disebabkan karena suatu kolom memiliki kapasitas volume injeksi sampel tertentu, jika volume sampel yang diinjeksikan melebihi kapasitas, maka pendeteksian suatu sampel tidak dapat maksimal [6]. Jadi dapat disimpulkan bahwa kolom yang digunakan hanya dapat menganalisa klorpirifos dengan volume injeksi sampel yang optimum yaitu 5 µL.




Gambar 2. Pengaruh volume injeksi sampel

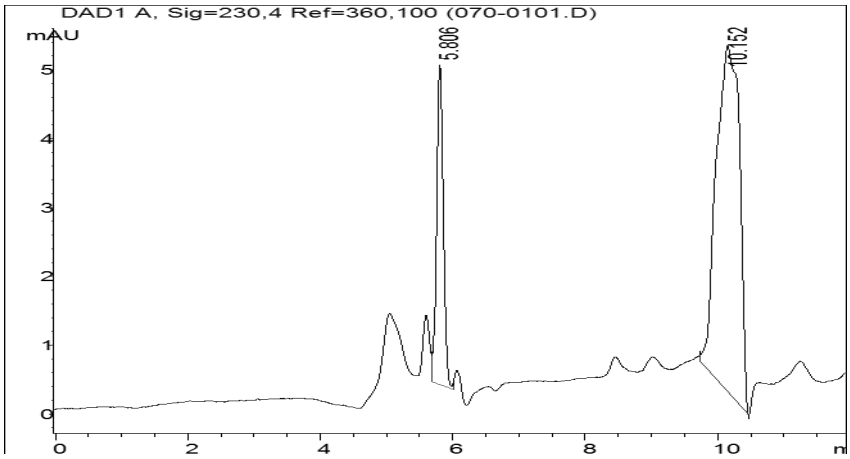
**Pengaruh Laju Alir Fasa Gerak**

Dalam pengukuran parameter ini digunakan komposisi fasa gerak dan volume injeksi sampel yang optimum hasil pengukuran sebelumnya, yaitu air:metanol dengan perbandingan 70:30 dan volume injeksi sampel 5 µL. Hasil Pengukuran dapat dilihat pada Gambar 3.




Gambar 3**.** Pengaruh Laju alir fasa gerak

Berdasarkan Gambar 3. diperoleh pada saat digunakan laju alir 0,3 mL/menit, kromatogram yang dihasilkan memiliki luas area yang lebih besar tetapi bentuk kromatogramnya tidak simetris, sehingga tidak dapat digunakan untuk analisa karena salah satu syarat penentuan yang baik memiliki waktu retensi yang cepat dan hasil yang akurat. Pada pengukuran ini diplih laju alir fasa gerak 0,5 mL/menit, karena menghasilkan kromatogram yang memiliki luas area yang paling baik dan waktu retensi yang diperoleh, stabil pada 3,509 menit.

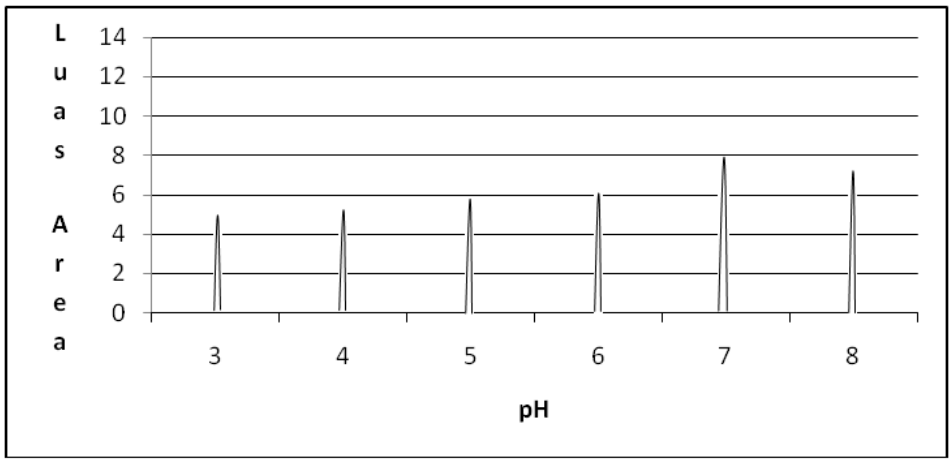



Gambar 4. Kromatogram dengan laju alir 0,3 mL/menit

**Pengaruh pH Fase Gerak**

Dalam pengukuran parameter ini digunakan komposisi fasa gerak, volume injeksi sampel dan laju alir fasa gerak yang optimum hasil pengukuran sebelumnya, yaitu air:metanol dengan perbandingan 70:30, volume injeksi sampel sebanyak 5 µL dan laju alir fasa gerak sebesar 0,5 mL/menit.

Fasa gerak yang digunakan diatur pada variasi pH 3 - 8. Jika pH < 3, ikatan silika dapat terputus (terhidrolisis), dan jika pH > 8, silika akan larut, karena silika dapat larut dalam suasana basa [6]. Pada Gambar 5. dapat dilihat bahwa perbedaan pH fasa gerak memberikan perubahan terhadap luas area kromatogram pada larutan standar klorpirifos. Luas area kromatogram yang paling besar dihasilkan pada fasa gerak pH 7 dibandingkan dengan fase gerak pH lainnya, sehingga dapat disimpulkan pH 7 adalah pH optimum untuk analisa klorpirifos




Gambar 5. Pengaruh pH fasa gerak

Dari hasil optimasi parameter HPLC untuk penetapan dapat dilihat pada Tabel 1.

**Penentuan Kinerja Analitik**

Setelah diperoleh hasil dari optimasi parameter kromatografik HPLC, kemudian dilanjutkan dengan penentuan kinerja analitik yang terdiri dari kebolehulangan, batas deteksi (LOD), linieritas, persentasi koefisien variansi (% KV) dan persentase perolehan kembali (% *recovery*).

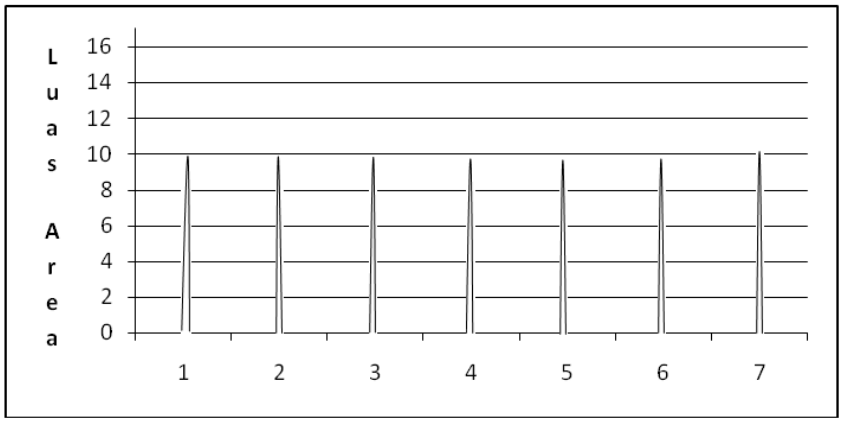
Tabel 1. Hasil pengukuran optimasi parameter kromatografik pada penentuan klorpirifos dengan HPLC

| **Parameter** | **Hasil Pengukuran** |
|---|---|
| Kolom | : Si-$C_{18}$ Shim-Pack VP-ODS (4,6 x 250 mm) |
| Komposisi Fasa Gerak | : air : metanol (70:30) |
| Volume Injeksi Sampel | : 5 µL |
| Laju alir fasa gerak | : 0,5 mL/menit |
| pH Fasa Gerak | : pH 7 (buffer fosfat) |
| Panjang gelombang | : 230 nm |
| Detektor | : UV |

**Kebolehulangan**

Kebolehulangan merupakan ukuran yang menunjukkan derajat kesesuaian/ kedekatan hasil antara hasil uji analisis yang dianalisis dengan cara yang sama. Pada penelitian ini, presisi ditetapkan berdasarkan keterulangan luas area kromatogram hasil analisa dengan tujuh kali pengulangan pada larutan standar. Perbedaan absolut dari data hasil analisa diharapkan berada dalam kisaran nilai kepercayaan 95% atau nilai relatif standar deviasi lebih kecil dari 5%. Untuk kriteria presisi dinyatakan dengan koefisien

variasi yaitu sebesar 2% atau kurang untuk konsentrasi larutan standar dengan konsentrasi ppm (mg/L) [7]. Hasil pengukuran dapat dilihat pada Gambar 6.

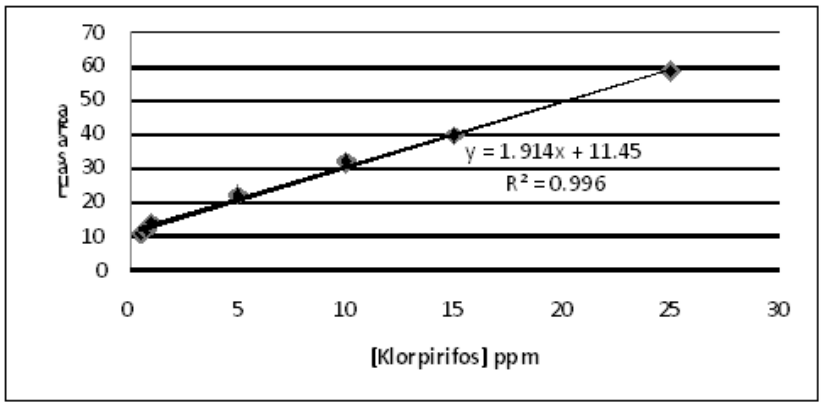



Gambar 6**.** Pengukuran kebolehulangan larutan standar klorpirifos 0,5 ppm

Kebolehulangan ditunjukkan dengan % KV (koefisien variansi). Hasil pengukuran ditunjukkan pada Gambar 6. Dari hasil penelitian yang diperoleh % KV untuk penentuan klorpirifos 0,5 ppm adalah 0.0664%. Dari hasil dapat dikatakan bahwa sistem operasional alat dan analisis memiliki nilai kebolehulangan yang baik terhadap metode dengan respon yang relatif konstan, sehingga hasil pengukuran memiliki nilai presisi yang memenuhi persyaratan.

**Penentuan Kurva Kalibrasi (Linieritas)**

Penentuan linieritas pada pembuatan kurva kalibrasi klorpirifos dilakukan pada kondisi optimum yang diperoleh pada optimasi parameter HPLC.




Gambar 7. Kurva kalibrasi larutan standar klorpirifos

Hasil pengukuran menunjukkan persamaan garis regrasi y = 1,914x + 11,45 dengan nilai koefisien korelasi $R^2$ = 0,996. Nilai regresi yang baik adalah > 0,99 [7][8], maka nilai ini telah memenuhi syarat digunakan untuk pengukuran analisis rutin.

**Limit Deteksi (*Limit of Detection, LOD*)**

Ketidakpastian terhadap analisis akibat pengukuran contoh uji yang sangat rendah, dapat teratasi dengan menentukan limit deteksi. Limit deteksi berguna dalam memastikan suatu respon yang ditimbulkan suatu análisis [8].

Penentuan limit deteksi pada percobaan ini berdasarkan pada pengukuran larutan standar sebanyak 7 kali dan ditentukan simpangan baku dari data yang ada sehingga dapat diketahui nilai deteksi dengan rumus yang ada. Dari hasil penelitian yang dilakukan untuk penentuan klorpirifos dengan metode HPLC diperoleh batas deteksi klorpirifos sebesar 0,44 ppm.

**Perolehan kembali (*% Recovery*)**

Salah satu syarat metode analisis yang baik adalah memiliki ketelitian atau akurasi yang tinggi. Parameter uji yang digunakan untuk menilai ukuran akurasi pada penelitian ini adalah parameter persentase perolehan kembali (% *recovery*). Pada penelitian ini, pengukuran persentase perolehan kembali dilakukan dengan

metode *spike,* dengan tujuan untuk melihat pengaruh matriks sampel berupa sayur-sayuran pada pengukuran konsentrasi klorpirifos.

Hasil pengukuran persentase perolehan kembali yang dihasilkan untuk penentuan klorpirifos dalam sayur-sayuran untuk 3 sampel berbeda, antara lain sampel Kubis sebesar 96%, sampel Kembang kol sebesar 103%, dan sampel sawi putih sebesar 98%.

Tabel 2. Konsentrasi klorpirifos dalam sayur-sayuran yang telah dikeringkan

| No. | Sampel | [Klorpirifos] µg/kg |
|---|---|---|
| 1. | Kubis | 0,131 ± 0,008 |
| 2. | Kembang Kol | 0,013 ± 0,005 |
| 3. | Sawi Putih | 0,109 ± 0,009 |

**Penentuan Konsentrasi Klorpirifos dalam Sayur-sayuran**

Tujuan penelitian ini adalah untuk menentukan konsentrasi klorpirifos dalam sayur-sayuran dengan menggunakan HPLC yang sebelumnya dioptimasi parameter kromatografiknya yang dilanjutkan dengan penentuan kinerja analitik sehingga diperoleh kondisi yang optimal untuk analisis. Kadar klorpirifos dari masing-masing sampel dapat dilihat pada Tabel 2.

Hasil penelitian menunjukkan bahwa residu klorpirifos dapat terdeteksi dalam sayur-sayuran, sehingga perlu menjadi pertimbangan bagi para konsumen dan pihak berwenang tentang keberadaan dan penggunaan senyawa ini.

## KESIMPULAN

Berdasarkan hasil penelitian yang telah dilakukan, maka dapat diambil kesimpulan bahwa tehnik HPLC dapat digunakan untuk menentukan residu klorpirifos dalam sayur-sayuran dengan ketepatan dan keakuratan yang tinggi, sehingga tehnik HPLC ini layak digunakan untuk analisis rutin senyawa klorpirifos dalam berbagai sampel.

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