Method Development for Pesticide Residue Analysis in Farmland Soil using High Performance Liquid Chromatography

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Abstract. A method for the determination of diazinon and chlorantraniliprole in soil samples has been developed. The analyte was extracted with acetonitrile from farmland soil sample. Determination and quantification of diazinon and chlorantraniliprole were performed by high performance liquid chromatography (HPLC) with an UV detector. Several parameters of HPLC method were optimized with respect to sensitivity, high resolution of separation, and accurate determination of diazinon and chlorantraniliprole. Optimum conditions for the separation of two pesticides were eluent composition of acetonitrile:water ratio of 60:40, 0.4 mL/min of flow rate, and 220 nm of wavelength. Under the optimum conditions, diazinon linearity was in the range from 1-25 ppm with R² of 0.9976, 1.19 mgL⁻¹ LOD, and 3.98 mgL⁻¹ LOQ; while the linearity of chlorantraniliprole was in the range from 0.2-5 mgL⁻¹ with R² of 0.9972, 0.39 mgL⁻¹ LOD, and 1.29 mgL⁻¹ LOQ. When the method was applied to the soil sample, both pesticides showed acceptable recoveries for real sample of more than 85%; thus, the developed method meets the validation requirement. Under this developed method, the concentrations of both pesticides in the soil samples were below the LOD and LOQ (0.577 mgL⁻¹ for diazinon and 0.007 mgL⁻¹ for chlorantraniliprole). Therefore, it can be concluded that the soil samples used in this study have neither diazinon nor chlorantraniliprole.

Keywords: HPLC, Pesticide, Diazinon, Chlorantraniliprole

1. Introduction

Pesticides Pesticides (herbicides, fungicides, insecticides or acaricides) have an important role in agricultural development and guarantees of increased agricultural production. The use of these chemical compounds affects soil, water, and food contamination, leading to the accumulation of contaminants into the environment that can enter the human food chain [1]. Pesticides are found in soil [2], which causes the accumulation of pesticide residue in the soil that takes a long time to degrade causing soil contamination and causing the decline of biodiversity of organisms in the soil [3]. Contact with the residue of the pesticides will cause adverse effects to health, including cancer, depression, respiratory disorders, neurologic effects and dermatological disease [3].

Diazinon is an organophosphate insecticide with molecular weight of 304.35 g/mol, with boiling point 83 - 94°C, vapor pressure of 4.6 x 10⁻⁵ mmHg at a temperature of 10°C, 1.4 x 10⁻⁴ mmHg at a temperature of 20°C, and 1.1 x 10⁻³ mmHg at 400°C, water solubility 0.004% at 20°C and dissolved in...
organic solvent. The diazinon structure with its chemical name O,O-diethyl-O-(2-isopropyl-6-methyl-4,4'-pyrimidyl) phosphorothioate. Diazinon absorbs UV light at a wavelength of 247 nm [4].

Chlorantraniliprole with a new action mode is called "Ryanodine Receptors Activators" [5] [6] and is a stomach poison and a contact poison. Symptoms indicated by insects due to the application of chlorantraniliprole insecticide are paralysis, stop eating and within a few days can lead to death [4]. Chlorantraniliprole with chemical name 3-bromo-N-[4-chloro-2-methyl-6 - [(methyl amino) carboryl] phenyl] -1- (3-chloro-2-pyridynil) -1H-pyrazole-5-carboxamide absorbs UV light at a wavelength of 260 nm [7].

The disadvantage of the liquid-solid extraction technique that used in the determination of residual pesticides in the soil are the use of large amounts of solvent and a long time to analysis, but it can be solved by other extraction methods namely Ultrasonic Solvent Extraction (USE) [8]. USE has several advantages: speed, selectivity, and low use of the solvent. USE increases the efficiency of extraction but because of its low selectivity it is followed by a clean up to obtain a clean extract [8]. For the detection and quantification of pesticide residues, one used high performance liquid chromatography (HPLC).

Chromatography is a method in which the mixed components are separated on the absorbent column in the flow system. For chromatographic separation, samples are included in the mobile phase flowing through the stationary phase [9]. HPLC was used for the analysis of some thermolabile or highly polar compounds and also on compounds with high molecular weight. The success of an analysis is also strongly influenced by the accuracy of the chromatographers in selecting and using column, the stationary phase, and the mobile phase [10]. The advantages of separation with HPLC compared to conventional methods include fast analysis times, low cost, and able to analyze compounds with low stability [11]. The method of analysis related to pesticide residue content ever done is the determination of chlorantraniliprole pesticide residue using solid phase extraction method (SPE) and HPLC using UV detector at 260 nm wavelength, this method has 200 pg detection limit and more than 97% recovery [7]. Determination of diazinon pesticide residues in water and soil samples is using HPLC using UV detector at 245 nm wavelength; this method has a quantification limit of 0.1 ng/ml water sample whereas in soil samples of 1 ng/ml and % recovery ranges 86 to 102% [12].

The research conducted optimization of eluent composition, flow rate, and wavelength. Furthermore, the validity test, consisting of linearity test, accuracy test, precision test, limit of detection (LOD), and limit of quantification (LOQ).

2. Methods

2.1 Chemicals

The materials used in this research were: a soil sample was taken from Bumiaji Sub-district, Batu City, Malang. 99% acetonitrile (Merck), standard diazinon pestanal™ 98.6% (Sigma-Aldrich), pestanal® 99.2% (Sigma-Aldrich) chlorantraniliprole standard. The tools that used in this research were: Genesys 10S UV-VIS spectrophotometer, ultrasonic cleaner (model: 008; unit size: 176 x 108 x 130 mm; tank size: 150 x 85 x 63 mm; volt: AC 220-240 V 50 Hz; ultrasonic power: 50 W; frekuensi: 40 kHz); timer: 0-30 min; full capacity: 800 mL), magnetic stirer, syringe pump and high performance liquid chromatography (Shimtzu Coorporation, Kyoto Japan) consisting of prominence degassing unit (DGU-20A5R), prominence liquid chromatography (LC-20AD), prominence comunication bus module (CBM-20A), prominence UV/VIS detector (SPD-20AV), column Lichrospher® 100 RP-8, 10 μm, 250 mm x 4 mm i.d.

2.2 Procedure

2.2.1 Preparation of standard solutions

The diazinon standard was prepared by dilution process using acetonitrile solvent until the standard solution of 25 mgL⁻¹ was obtained. The chlorantraniliprole standard was prepared by dilution using acetonitrile solvent until a chlorantraniliprole solution was obtained at a concentration of 5 mgL⁻¹. The standardized was 25 mgL⁻¹ standard diazinon and 5 mgL⁻¹ standard chlorantraniliprole.
2.2.2. Optimization
This method was optimized under condition of composition of the mobile phase of acetonitrile/water from 50-80% for isocratic elution system, flow rate (0.2; 0.4; 0.6; 0.8; 1.0 mL/min), and wavelength UV detector (220-260 nm).

2.2.3. Sample preparation
The soil sample was taken from the apple farm area of Bumiaji Sub-district, Batu City. The soil was taken at 0-10 cm deep, sieved and stored in airtight plastic at ± 4°C until the soil sample would be analyzed. The soil sample weighed 20 g and 60 ml of acetonitrile was added to the soil. The solution was put in a 100 ml beaker glass. It was stirred for 1 hour and sonicated for 2 minutes. The extract was filtered using 0.2 μm filter paper.

2.2.4. Optimization of LC-UV method
Each standard solution of prepared pesticide was taken as ± 1 mL using a 1 cc syringe. It was subsequently injected at HPLC with the condition of separation HPLC instrument as follow:

| Parameter       | Condition                                    |
|-----------------|----------------------------------------------|
| Temperature     | room temperature                             |
| Column          | Lichrospher® 100 RP-8, 10 μm, 250 mm x 4 mm i.d |
| Flow rate       | 0.4 mL/min                                   |
| Detector        | UV 240 nm                                    |
| Mobile phase    | acetonitrile:water (80:20), (70:30), (60:40), (50:50) |

The optimum eluent composition of the separation result was determined by the values of R, N, k’, and α of the formed chromatograms.

Determination of optimum flow rate was done with separation condition of HPLC instrument, with selected eluent composition (optimum condition), with variation of flow rate (0.2; 0.4; 0.6; 0.8; 1.0 mL/min). The optimum flow rate of the separation results was determined by the values of Rs, N, k’, and α of the formed chromatograms.

Determination of the optimum wavelength was performed by the separation condition of the HPLC instrument, with eluent composition and the selected flow rate (optimum condition), with the variation of wavelength (220, 230, 240, 250, and 260 nm). The optimum wavelength of the separation result is determined by the formed chromatogram.

For qualitative and quantitative analysis, the dilute extract soil was injected in LC-UV system under optimum mobile phase of acetonitrile:water (60:40), optimum flow rate (0.4 mL/min), and maximum wavelength (220 nm). Qualitative analysis was done by comparing the retention time of standard MET with the retention time of peaks form Myrmeleon sp. Chromatogram and by spiking method, where the diazinon and chlorantraniliprole may be determined by increasing peak area by standard diazinon and chlorantraniliprole injection. Spiking method was conducted by injecting 20 μL of diazinon standard and 40 μL of chlorantraniliprole standard into 2 mL of soil extract followed by homogenization with sonication for 300 seconds, and then filtration through a 0.2 μm filter paper before injection into the LC-UV system. The presence of diazinon and chlorantraniliprole were detected by the peak with higher intensity (area) compared to chromatogram peak from the un-spiked sample. Quantitative analysis of diazinon and chlorantraniliprole in the soil extract were done using spiking. The concentration of diazinon and chlorantraniliprole were determined by comparing the peak area of diazinon and chlorantraniliprole before and after spiking with a known concentration of diazinon and chlorantraniliprole standard solution.

3. Results and Discussion

3.1 Optimization of LC-UV method
The chromatogram of diazinon and chlorantraniliprole separation resulted in four variations of acetonitrile:water composition can be seen in Figure 1. Figure 1 showed that the more acetonitrile solvent used, the faster the retention time of the compound. This was caused by nonpolar columns and
polar solvents so that the more non-polar compounds would be retained longer in the column. At 60:40 of mobile phase compositions, both compounds separated well at the retention time of each compound, chlorantraniliprole 9.8 and diazinon 18.64 and a short retention time of fewer than 20 minutes.

**Figure 1.** Chromatogram Standard of Chlorantraniliprole (1) dan Diazinon (2)
(Separation conditions: Lichrospher® 100 RP-8, 10 μm, 250 mm x 4 mm i.d; V.sampel 2 μL; Flow rate: 0.4 mL/min; λ = 240 nm)

Figure 2 showed the effect of the flow rate on the retention time of diazinon and chlorantraniliprole compounds.

**Figure 2.** Chromatogram Standard of Chlorantraniliprole (1) dan Diazinon (2)
(Separation conditions: Lichrospher® 100 RP-8, 10 μm, 250 mm x 4 mm i.d; V.sample 2 μL; Composition of Acetonitrile:Water (60:40); λ = 240 nm)

Figure 2 showed that faster the flow rate caused smaller standard peak area. So it may be determined the optimum flow rate conditions for the separation of diazinon and chlorantraniliprole was 0.4 mL/min. Where the fast analysis time is below 20 minutes and the large peak compound area was 550134 for chlorantraniliprole and 427586 for diazinone.

Figure 3 showed the influence of wavelength on peak intensity diazinon and chlorantraniliprole.
Figure 3. Chromatogram Standard of Chlorantraniliprole (1) dan Diazinon (2) (Separation Condition: Column Lichrospher® 100 RP-8, 10 μm, 250 mm x 4 mm i.d; V.sample 2 μL; Composition of Acetonitrile:Water (60:40); Flow rate: 0.4 mL / min)

From Figure 3 may be seen that the optimum wavelength for diazinon as well as chlorantraniliprole was at 220 nm of wavelength because it exhibited high sensitivity compared to other wavelengths so that both components (diazinon and chlorantraniliprole) may be detected.

3.2 Validation method

Validation method was done in optimum condition. Standard curve diazinon and chlorantraniliprole may be seen in Figure 4. Method validation obtained diazinon linearity was in the range from 1-25 mgL$^{-1}$ with R$^2$ of 0.9976, 1.19 mgL$^{-1}$ LOD, and 3.98 mgL$^{-1}$ LOQ; whilst the linearity of chlorantraniliprole was in the range from 0.2-5 mgL$^{-1}$ with R$^2$ of 0.9972, 0.39 mgL$^{-1}$ LOD, and 1.29 mgL$^{-1}$ LOQ. When the method was applied to the soil sample, both pesticides showed acceptable recoveries for real sample of more than 85% (Table 1) thus, the developed method met the validation requirement.

Figure 4. Standard Curve Diazinon (a) and Chlorantraniliprole (b)

3.3 Analysis of pesticide

Qualitative analysis of diazinon and chlorantraniliprole in soil samples using spiking method was by adding standard diazinon and chlorantraniliprole solutions which were known to concentrate into soil samples. Figure 5 showed that there is no peak increase at the same retention time was the standard retention time is dispersed into the soil sample extract. Whereas after a quantitative analysis of the
diazinon and chlorantraniliprole in soil samples, the concentrations of both pesticides in the soil samples were below the LOD and LOQ (0.577 mgL\(^{-1}\) for diazinon and 0.007 mgL\(^{-1}\) for chlorantraniliprole). Therefore, it can be concluded that the soil samples used in this study have neither diazinon nor chlorantraniliprole.

Table 1. % Recovery of Pesticides in Soil (n = 3)

| Compound      | Soil sample | Concentration added (mgL\(^{-1}\)) | Concentration measured (mgL\(^{-1}\)) | % recovery |
|---------------|-------------|------------------------------------|--------------------------------------|------------|
| Diazinon      | A           | 0                                  | 0,17                                 | -          |
|               | B           | 5                                  | 0,19                                 | 87,73 %    |
| Chlorantraniliprole | A    | 0                                  | 0,06                                 | -          |
|               | B           | 1                                  | 0,07                                 | 95,65 %    |

![Figure 5. The Chromatogram of The Soil Sample, The Soil Sample Spiked with 5 mgL\(^{-1}\) Diazinon, and The Soil Sample Spiked with 1 mgL\(^{-1}\) Chlorantraniliprole](image)

4. Conclusion
Optimum conditions for the separation of two pesticides were eluent composition of acetonitrile:water ratio of 60:40, 0.4 mL/min of flow rate, and 220 nm of wavelength. Under the optimum conditions, diazinon linearity was between 1 and 25 mgL\(^{-1}\) with \(R^2\) of 0.9976, 1.19 mgL\(^{-1}\) LOD, and 3.98 mgL\(^{-1}\) LOQ; whilst the linearity of chlorantraniliprole was between 0.2 and 5 mgL\(^{-1}\) with \(R^2\) of 0.9972, 0.39 mgL\(^{-1}\) LOD, and 1.29 mgL\(^{-1}\) LOQ. When the method is applied to the soil sample, both pesticides show acceptable recoveries for real sample of more than 85%: thus, the developed method meets the validation requirement. The concentrations of both pesticides in the soil samples are below the LOD and LOQ (0.577 mgL\(^{-1}\) for diazinon and 0.007 mgL\(^{-1}\) for chlorantraniliprole). Therefore, it can be concluded that the soil samples used in this study have neither diazinon nor chlorantraniliprole.

References
[1] P. Tette, L. Guidi, M. Gloria, and C. Fernandes (2016), Pesticides in Honey: A Review on Chromatographic Analytical Methods, *Talanta*, 149, 124–141.
[2] C. Sanchez-Brunete, B. Albero, and J. Tadeo (2004), Multiresidue Determination of Pesticides in Soil by Gas Chromatography-Mass Spectrometry Detection, *J. Agric. Food Chem.*, 52, 1445–1451.
[3] Indrianingsih, A., Nisa, K., Wahono, S. (2007), Analisis Residu Pestisida dalam Tanah dan Umbi Bawang Merah di Lahan Pasir Sanden, Bantul, Yogyakarta dengan Kromatografi Gas, di Seminar Nasional Rekayasa Kimia Dan Proses 2007, 181–185.
[4] E. Hartanti, F. Mahatmanti, and E. Susatyo, (2012), Sintesis Kitosan_Bentonit serta Aplikasinya Sebagai Penurun Kadar Insektisida Jenis Diazinon,” *Indones. J. Chem. Sci.*, 1, 111–115.
[5] Cordova, D., Benner, E. A., Sacher, M. D., Rauh, J. J., Sopa, J. S., Lahm, G. P., Tao, Y.
(2006), Anthranilic Diamides: A New Class of Insecticides with A Novel Mode of Action, Ryanodine Receptor Activation, *Pestic. Biochem. Physiol.*, 84, 196–214.

[6] Lahm, George P., Selby, Thomas P., Freudenberger, John H., Stevenson, Thomas M., Myers, Brian J., Seburyamo, G., Smith, Ben K., Flexner, L., Clark, Christopher E., Cordova, D. (2005), Insecticidal Anthranilic Diamides: A New Class of Potent Ryanodine Receptor Activators,” *Bioorg. Med. Chem. Lett.*, 15, 4898–4906.

[7] P. Xu, Y. Ren, Z. Zhou, A. Liu, and H. Zhang (2010), Determination of Chlorantraniliprole in Vegetables, Fruits, and Grains by SPE, *Chromatographia*, 72, 763–766.

[8] C. Lesueur, M. Gartner, A. Mentler, and M. Fuerhacker, (2008), Comparison of Four Extraction Methods for The Analysis of 24 Pesticides in Soil Samples with Gas Chromatography-Mass Spectrometry and Liquid Chromatography – Ion Trap – Mass Spectrometry, *Talanta*, 75, 284–293.

[9] Moldoveanu, S., David, V., (2013), Essentials In Modern HPLC Separations, USA: Elsevier.

[10] Rouessac, F. and Rouessac, A., (2007), Chemical Analysis Modern Instrumentation Methods and Thecniques, Edisi kedua, Francis.

[11] Nurhamidah, (2005), Penentuan Kondisi Optimum HPLC untuk Pemisahan Residu Pestisida Imidakloprid. *Ilmu-Ilmu Pertanian Indonesia*, 7(2), 87–93.

[12] M. E. Sánchez, R. Méndez, X. Gómez, and J. Martin-Villacorta, (2003), Journal of Liquid Chromatography & Related Determination of Diazinon and Fenitrothion in Environmental Water and Soil Samples by HPLC, *Liq. Chromatogr. Relat. Technol.*, 26, 483–497.