Determination of carbamate insecticide in soil by SPE reversed-phase high-performance liquid chromatography (RP-HPLC)

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Abstract. Carbamate pesticides usages in agriculture are increasing significantly compared with other organohalogen pesticides, due to carbamate compounds have been considered to be stable compounds in the environment in term of their application for preventing leaves and fruits from plant disease. A rapid multi-residue method had been developed for detecting carbofuran and carbaryl, types of carbamate insecticide, in agriculture soil. This method was based on shaking extraction followed by solid phase extraction (SPE) of soil samples. Residue content of this carbamate insecticide was analyzed by using reversed-phase high-performance liquid chromatography with UV detection. Carbofuran and carbaryl were separated by using a C18 column and acetonitrile-water as the mobile phase. In this study, soil samples were taken from three locations (Pangalengan, Lembang and Cisarua) for detecting the content of carbamate insecticide. Precision test of Pengalengan soil sample in wet season for carbofuran and carbaryl were obtained at 5.42% and 6.75%, respectively. Recovery testing was carried out by using the fortification technique in which respectively 0.5 and 1.0 mg/kg content of carbofuran and carbaryl were spiked into the samples. The average recovery was in the range of 80.53 to 82.06 % and the standard deviation was between 3.78 and 4.51% for carbofuran and carbaryl, respectively. The calibration curve provided linear results in the concentration range of 0.05 to 10 µg/mL and 0.025 to 11.2 µg/mL with a correlation coefficient of 0.9999 and 0.9999 for carbofuran and carbaryl, respectively. On average, carbofuran and carbaryl in the soil were detected at 0.3350 and 0.2958 µg/g, respectively, for both samples from Pangalengan and Lembang during dry season while neither of these compounds was detected in Cisarua and Pangalengan during the wet season. This shows that carbamate insecticides had been washed out form soil by rainwater during the wet season or through degradation pathways in the environment.

1. Introduction

Pesticide residues are known as toxic and dangerous chemical compounds and have a long lifetime effect if present in the environment due to their stability and movability [1]. Some national and international regulations have been set out the threshold of pesticide residues content in the food commodity and environment, among them are Agency for Toxic Substances and Disease Registry and the United States Environmental Protection Agency (US-EPA) which list 20 dangerous compounds [2,3], and the European Chemicals Agency (ECHA) that proposes 16 types of compounds categorized as Substances of Very High Concern (SVHC) [4,5]. No doubt, the contaminant agents such pesticide residues potentially endanger not only human but also biota in the ecosystem, so the monitoring and
controlling of these contaminant agents need to be done seriously and consistently. Carbamate pesticides usages in agriculture are increasing significantly compared with other organohalogen pesticides, due to carbamate compounds have been considered to be stable compounds in the environment in term of their application for preventing leaves and fruits from plant disease attack [6,7].

In general, measurement of trace compounds such as pesticide residues has high difficulty due to time consumption and a long procedure causing losses of the analyte. Since method based on the derivatisation of carbamates to thermally stable products have several limitations that often reduce their sensitivity in Gas Chromatography method, High-Performance Liquid Chromatography (HPLC) method has become the preferred choice for carbamate determination. An HPLC method for the simultaneous detection of ten carbamate pesticides in water and soil has shown a good recovery of carbamate residues from water and soil samples. In addition, this method is highly selective for determining residues of some oximes, N-methyl carbamates, phenyl-N-methylcarbamates and N-phenylcarbamates, even though the extraction process is considered takes time, using conventional column chromatography before injecting into UV-HPLC equipped with ODS reversed-phase column [8]. Fluorescence detector was reported to improve the sensitivity of detection for analyzing carbamate residues [9]. Post column derivatisation techniques in HPLC method have been developed all over the world for increasing the sensitivity of the analysis. Conventional extraction using mechanical shaking also has been done in routine analysis for increasing the sensitivity. Supercritical fluid extraction (SFE) has been successfully applied to soil analysis as a practical alternative to conventional methods. A previous study that used sonication-assisted extraction (SAESC) for the analysis of carbamate insecticides (oxamyl, methomyl, propoxur, carbofuran, carbaryl and methiocarb) in the soil before injecting into RP-HPLC-FI had shown a good result for determining those carbamate insecticides [10]. This study described the development of an analytical method for detecting pesticide residues with high accuracy and precision. We also demonstrated the simple preparation of samples for detection the carbamate pesticides in the soil that is easy to be applied in the laboratory, faster and has less consumption of dangerous chemical reagents.

2. Experimental

2.1. Soil samples

Soil samples were taken from three locations namely Pulosari Village in Pangalengan, Bandung District; Sunten Jaya Village in Lembang, West Bandung District; and Jambu Dipa Village in Cisarua, West Bandung District. These areas are central of tomato, potato, cabbage, cucumber, cauliflower, broccoli and the other horticulture products. Farmers always use carbamate pesticides for protecting their agriculture products from the plant disease attack. In Pulosari Village, Pangalengan, the soil was taken in the wet and dry season while in other villages, the soil was taken only in dry season. Several soil samples were taken randomly and mixed all together with the square cone method based on Regulation of the Minister of State Environment No. 07 2006 about Procedures for Measuring Raw Criteria Land Damage for Biomass Production [11].

| Location    | pH    | Temperature | Moisture | Light |
|-------------|-------|-------------|----------|-------|
| Pangalengan | 7.0   | 22 °C       | Dry      | Low   |
| Lembang     | 5.5   | 21 °C       | Wet      | Low   |
| Cisarua     | 6.5   | 24 °C       | Dry      | Low   |

The physical-chemical properties of the soil (pH, temperature, moisture and light) are presented in Table 1. Soil samples were taken at 10-20 cm depth from the soil surface. The soil was sieved (40 mesh) and kept in cold room at the temperature of ± 15 °C. Before the analysis, the sample was
conditioned to reach room temperature. This procedure was taken from literature [11–13] and had been modified for treatment of the soil samples and analysis of carbaryl and carbofuran.

2.2. Chemicals and reagents
Carbofuran and carbaryl standards were obtained from Sigma-Aldrich and dissolved in methanol from Merck. Acetonitrile of analytical grade (for extraction) and chromatography grade (for eluent) was obtained from Merck. SPE (solid phase extraction) Sampliq C-18 type was obtained from Agilent Technologies. Syringe filter PTFE was used from the membrane solution.

2.3. Instrumentation
Hitachi HPLC-RP equipped with D-7500 integrator, L-7100 pump and UV detector on wavelength 275 nm was used, as well as C-18 (diameter 3.9 x 300 mm) column from Waters. Hettich Zentrifugen EBA-12 centrifuge was used for sample preparation. Field instrument device was used for measuring pH, moisture, temperature, and light intensity of soil.

2.4. Soil sample extraction
Soil sample (5 g) was extracted with acetonitrile (10 mL) for 2 hours in a shaking machine. Centrifugation was carried out for 3 minutes at 3000 rpm to obtain an aliquot, which is subsequently transferred into a syringe filter for obtaining a clear liquid. This procedure was performed twice and all clear liquid was mixed all together and transferred into the SPE [12].

2.5. Solid Phase Extraction (SPE)
Solid phase extraction containing C-18 was conditioned with methanol and aquadest, then loaded with clear liquid and followed by elution with methanol directly in this column [13].

2.6. Preparation of standard solution
Stock solution for carbofuran and carbaryl in methanol were prepared at the concentration of 1 mg/mL each. From the stock solution, a 100 µg/mL solution was prepared in a 5 mL volumetric flask. This solution was further diluted to the concentrations of 0.025; 0.05; 0.01; 0.1; 2.0; 2.5; 5.0; and 10 µg/mL in methanol. The stock solution was also used for fortifying the soil samples.

2.7. Analysis of soil sample
Soil sample that has been extracted as described above was transferred into the SPE and then injected into the HPLC. Carbofuran and carbaryl peaks were identified based on the retention time. Pesticide residues were determined by plotting the width area against the calibration curve of each compound.

3. Results and discussion
The peak of each compound can be seen in Figure 1, which shows a very clear difference between carbofuran and carbaryl at the retention time 9.26 minute and 10.30 minute for carbofuran dan carbaryl, respectively. Both peaks provided a good resolution due to there was a distinct separation on peak bases. Methanol as a solvent was also shown at retention time 3.42 minute. With relation to this result, the analysis of carbofuran and carbaryl can be performed by using the reverse phase C-18 and acetonitrile-water ratio of 45:55 with UV-HPLC.

Analysis of soil sample from Pulosari village in the Pangalengan in the wet season did not show any result for carbofuran and carbaryl compounds (Figure 2). This probably indicated that these compounds had been washed out by rainwater. These compounds, carbofuran and carbaryl, and their metabolites are highly soluble in water, and their stability under certain environmental condition has made them a serious threat in the water body. In relation to this result, this soil sample was fortified with carbofuran and carbaryl at 0.5 µg/g and 1.0 µg/g, respectively, and analyzed further with UV-HPLC without using SPE (Figure 3). Its recovery testing varied between 80-82 % and standard deviation (SD) between 3.7 and 7.3% (Table 2). This result was more or less similar with other studies
and the recovery values were still in the range of 80-110% for unit spiking 100 µg/g to 10 µg/g as mentioned in AOAC [15].

![Standard chromatogram of carbofuran and carbaryl.](image1)

**Figure 1.** Standard chromatogram of carbofuran and carbaryl.

![Chromatogram of soil sample without fortification.](image2)

**Figure 2.** Chromatogram of soil sample without fortification.

| Compound  | Recovery (%)<sup>a</sup> (0.5 µg/g) | Recovery (%)<sup>a</sup> (1 µg/g) |
|-----------|-----------------------------------|-----------------------------------|
| Carbofuran| 82.08 ± 5.39                      | 80.48 ± 3.78                      |
| Carbaryl  | 80.53 ± 7.31                      | 82.06 ± 4.51                      |

<sup>a</sup>Results are the mean of five replicates ± SD
Figure 3. Chromatogram of 0.5 µg/g fortified soil sample from Pangalengan without SPE.

The coefficient of variance (CV) for repeatability testing of carbofuran and carbaryl was 5.42 and 6.67%, respectively, less than Horwitz CV that was 13.46 and 13.42%. This result shows that this method was excellent for analysis of these compounds. Testing was also done by using SPE with this soil sample as presented in Figure 4. The chromatograms show significant differences between fortified soil sample which did not use SPE (Figure 3) and those which used SPE (Figure 4). On Figure 3, the sharp peak appeared at the retention time 7.92 minute and this peak also appeared in Figure 2 for soil sample without fortification, suggesting this peak was matrices or another compound in the soil. In Figure 4, the peak of matrices disappeared after passing the SPE, because C-18 column in SPE worked selectively where only certain analytes, carbofuran dan carbaryl, were detected. This also shows that SPE played a significant role in cleaning matrices up. In addition, the function of SPE was to concentrate analytes and reduce usage of organic solvent.

Figure 4. Chromatogram of fortified soil sample use SPE.
Table 3. Retention times ($t_R$), method detection limit (MDL), and calibration data.

| Compound   | $t_R$ (min) | MDL ($\mu$g/g) | Correlation coefficient ($r$) | Equation                          |
|------------|-------------|----------------|------------------------------|-----------------------------------|
| Carbofuran | 9.36        | 0.10           | 1.000                        | $y = 1.46 \times 10^4 x + 4.22 \times 10^2$ |
| Carbaryl   | 10.44       | 0.10           | 1.000                        | $y = 3.43 \times 10^4 x + 2.11 \times 10^3$ |

Detector responses were linear at the concentration range 0.05 to 10 $\mu$g/mL for carbofuran and 0.025 to 11.2 $\mu$g/mL for carbaryl. The coefficient of correlation for these two compounds was in the range 0.9999 to 1.000 (Table 3). Method detection limit (MDL) was 0.10 $\mu$g/g, which shows a high sensitivity result compared with the MDL of 0.14 mg/L found in a previous study [12], while the limit of quantitation (LOQ) was 0.50 $\mu$g/g.

Figure 5. Chromatogram for carbaryl pesticide from soil sample (a) Pangalengan, (b) Lembang, both in the dry season.

Measurement of carbofuran and carbaryl was done for soil samples from Pengalengan, Lembang and Cisarua in the dry season. Soil samples from Pengalengan and Lembang contained 0.3350 and 0.2958 $\mu$g/g carbaryl, while carbofuran was not detected (Figure 5). This result suggests that carbaryl was harder to degrade than carbofuran. This result was opposite to a previous study which shows that carbaryl is more degradable than carbofuran in distilled water. In fact, carbaryl is a very unstable compound which can be rapidly hydrolysed in aqueous medium [16]. Probably, the pesticide used by the farmers was added with buffer in practical uses in order to increase its stability and reducing hydrolysis in an aqueous medium.

Meanwhile, no carbofuran and carbaryl were detected in tomato plantation at Cisarua, which might be attributed to degradation of carbofuran and carbaryl in the soil. Several factors of degradation were reported such as hydrolysis, photolysis and microbial transformation, while 1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and 3-hydroxy, 3-keto derivatives have been reported as degradation products of carbaryl and carbofuran [17]. Another possibility is of the presence of earthworms which absorb carbamate pesticide since these earthworms are being used for toxicity testing of carbofuran and carbaryl in the soil [18].

4. Conclusion
Results obtained in this study show that the RP-HPLC–UV method with solid phase extraction, allows carbamate analysis in soil at low levels. The proposed procedure is a rapid and sensitive method based on the shaking extraction of soil samples using acetonitrile as an extracting solvent. This technique provides a good response of linearity, high precision and low detection limits. The LOQ for carbamates in soil is 0.50 $\mu$g/g. Analysis of pesticides can be carried out in a short time and consume less of organic solvent. A recovery study of carbamates can provide tolerance values within the allowable limit.
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