Determination of carbofuran in a river water sample using LC-MS/MS

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Abstract. A study on the presence of carbofuran was conducted in Cikapundung river from an agricultural zone of the Lembang, West-Java, Indonesia. The present study aimed to determine the trace carbofuran in river water using LC-MS/MS. Extraction and purification of carbofuran was carried out simultaneously by Solid Phase Extraction (SPE) system with C18 sorbent. The procedure was evaluated by carbofuran recovery determination. Recovery was studied by spike technique with the addition of 2 and 5 ng/g carbofuran in river water sample. The recovery were of 83.87 and 115.88 % with relative standard deviation (RSD) of 6.28 and 0.47 % respectively. Carbofuran contained in Cikapundung river water samples was of 0.2898 ng/g.

1. Introduction

Indonesia is one of the agrarians country. It is well known that the use of large quantities of pesticides in agricultural activities is one of the main causes of surface and ground waters pollution. Consequently, very strict program is required to control and monitor the levels of these contaminants.

One source of drinking water for the rural areas around Lembang generates from Cikapundung river. However, it may contains carbofuran from Furadan insecticide commonly used by farmers. Therefore analyzing carbofuran contained in Cikapundung river water is indispensable. Based on Ministry of Health Regulation No.492/Menkes/Per/IV/2010 of drinking water quality requirement, carbofuran contained in drinking water should be less than of 7 ng/g, which was the legal maximum concentration permitted [1].

Since the concentrations of the compounds was normally low in ng/g or less as well as due to the low detection levels required by regulatory bodies and the nature of the matrices in which the target compounds was present, efficient sample preparation and trace-level detection and identification are important aspects of analytical methods. The analytical methods with less quantity of solvents, easy and rapid to execute are required to monitor the widespread distribution of trace levels of pesticides [2].

Most carbamates are thermally unstable chemical. Hence, it is necessary to analyze by liquid chromatography (LC) rather than by gas chromatography (GC). LC with variety of detection system can be done such as LC-post column fluorescence detector system and LC-diode array UV detector system. LC-UV detection low sensitivity compare with fluorescent derivates. Derivatization with o-phtalaldehyde (OPA) is the basis of the LC-fluorescence method which has been accepted as a standard protocol by several official organizations, including Environmental Protection Agency (EPA) and Association of Official Analytical Chemist (AOAC) [3,4]. However, post column fluorescence requires o-phtalaldehyde (OPA) for derivatization [5]. Another LC with post column chemiluminescence (CL) detection also applies luminol for reaction. This method is based on the enhancing effect of these analytes on the CL emission generated by the oxidation of luminol with potassium permanganate in alkaline medium. Nowadays, the on-line combination of liquid chromatography and mass spectrometry (LC-MS) occupies an important place in the analysis of...
pollutants since this method provides for identification of thermally labile and polar pesticides at trace levels [6,7].

Extraction and purification were crucial step for sample preparation. Solid-phase extraction (SPE) used off-line or on-line is useful for trace determination of pesticides in water samples. SPE is already a well-established and routine technique. Presenting itself as a good alternative for the traditional techniques based on liquid–liquid extraction (LLE). LLE was needed a chemicals such as dichloromethane (DCM), methylene chloride, which was high consumption of solvent, which are often hazardous, which render them expensive or time consuming and organic solvent waste problem [5-9].

The concept of green chemistry emerged in the 1990s with the aim of minimizing the environmental impact of chemical activities. The basic principles to which a chemical procedure should correspond in order to be recognized as environmentally friendly (green) are reviewed. An important part of the green chemistry philosophy is the need to develop and adopt green analytical techniques and procedures. The key goals to be achieved in greening analytical methods are elimination or reduction of the use of chemical substances (solvents, reagents, preservatives, additives for pH adjustment and others); minimization of energy consumption; proper management of analytical waste; and increased safety for the operator. Most of these issues require reductions e.g., sample number, reagents, energy, waste, risk and hazard [10-15].

The objective of this work was to study of sample preparation and analysis of carbofuran in water sample based on analytical green chemistry (GAC). The analytical method then was applied to sample water from Cikapunung river as a source for drinking water.

2. Experimental
2.1. Standard and reagents
Carbofuran was supplied from Hunan Research Institute of Chemical Industry, HRICI – Changsha, PR. China, its purity of 99.7%. Stock standard solution was prepared by dissolving 10 mg of carbofuran in 10 g methanol. The working standard solution was prepared by dilution of the stock solution in methanol at concentration from 2 ng/g to 40 ng/g. The LiChrosolv-grade methanol and acetonitrile were supplied by Merck. SPE C18 cartridge was used for this study.

2.2. Source, collection and sample treatment
Water samples were collected from Cikapundung river, Eest Java, Indonesia on March which was rainy season. Freshly water sample was spike with carbofuran standard solution so that the concentration analyte in sample was 2 and 5 ng/g, respectively. To remove the suspended particulate matter, the spike sample was filtered thorough Whatman filter paper No.1 with a Buchner funnel and vacuum. Prior to analysis the filtered spike sample was stored in the dark glass bottle at 4°C.

2.3. Sample preparation
Extraction and purification of carbofuran was done simultaneously using Solid-phase extraction (SPE) with C18 as a sorbent. SPE cartridges was conditioned with 3 ml of methanol and 3 ml of deionized water. As many as 5 g of the spiked river water samples was applied to the cartridge, then was eluted by 5 g methanol. The eluent then was filtered thorough 0.45 µm membrane and analysis using LC-MS/MS. The blank sample was prepared with the same procedure without spiking with 3 replications.

2.4. Instrument and chromatographic condition
The instrument was LC-MS/MS 3200 QTRAP Triple Quadropole. Analysis carbofuran was performed on a Zorbax XDB-C8, 4.6 x 50 mm, 3.5 µm particle. The total flow rate of eluent A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) was 0.35 ml/min. The profile of gradient was 0-1.5 min/90% A/10% B, 1.5 – 5 min/10% A/90% B, 5-8 min/10% A/ 90% B, 8 – 8.5 min/90% A/10 B, 8.5 – 10 min/90% A/10% B. The analysis was performed at ambient temperature and injection volume was of 10 µl.
3. Result and discussion

3.1. Qualitative analysis
Molecular formula of carbofuran was C_{12}H_{15}NO_{3} and its molecular weight is of 221. Fragmentation schemes for carbofuran is shown in Figure 1 whereas ion chromatogram peak which retention time (tR) of 6.57 min is shown in Figure 2. The Multiple Reaction Monitoring (MRM) mode for carbofuran was precursors ion (m/z, Q1 mass) of 222.1 and products ion (m/z, Q3 mass) were 165.2 and 123.0, respectively. The isolated precursor and product ion in MRM using energy profile were De-clustering Potential (DE, volt) of 30.0, Entrance Potential (EP, volt) of 10.0, Collision Energy (CE, volt) of 17 and 29, Collision Exit Potential (CXP, volt) of 3.0.

![Fragmentation schemes of carbofuran](image-url)

Figure 1. Fragmentation schemes of carbofuran [8].

3.2. Extraction and purification by SPE- C18
Spike river water sample with carbofuran was applied to the SPE. Filtrate from SPE was then analyzed using LC-MS/MS. The result of the analysis showed that no carbofuran was identified. The results indicated that carbofurans were absorbed to C18 as an absorbent.

3.3. Recovery and accuracy
Accuracy was calculated of the known concentration level by comparing the measured concentration with the spike concentration as the reported method [10]. Sample spike at 2 and 5 ng/g concentration with number replicate of 3. The average result of recovery obtained was of 83.87 and 115.88%. These values was considered acceptable and good result since a 70 – 130% recoveries were approved [4,5]. Precision was evaluated in term of repeatability and expressed as relative standard deviation (RSD, %). The result of precision were 6.28 and 0.47% respectively, indicating good repeatability.
Figure 2. Extracted ion chromatogram (XIC) carbofuran standard solution of 25 ng/g.

Table 1. Experiment summary of recovery and precision.

| Replication | Spike conc, ng/g | Recovery, % | Spike conc, ng/g | Recovery, % |
|-------------|------------------|-------------|------------------|-------------|
| 1           | 1.9840           | 89.95       | 4.9581           | 115.2579    |
| 2           | 1.9824           | 80.88       | 4.9580           | 116.1623    |
| 3           | 1.9849           | 80.78       | 4.9555           | 116.2205    |
| Average     | 1.9842           | 83.8678     | 4.9581           | 115.8803    |
| Standard Deviation, SD | 5.2643 | 0.5397 |
| Rel. SD, %  | 6.28             | 0.47        |

3.4. Application of a qualitative analysis
The method was applied to water sample which was collected from Cikapundung river in rainy season on March of 2016. Concentration of carbofuran obtained was of 0.2098 ng/g was lower than requirement for water drinking of 7 ng/g. Analytical method was sensitive enough to detect the carbofuran. Water from Cikapundung river was safe for a source of drinking water. In this study, the concept of green analytical chemistry (GAC) has been done. Analysis of carbofuran using LC-MS/MS required mobile phase of 0.35 ml/min. Extraction and purification were required only as much as 5 g methanol. Flow rate and gradient system of mobile phase as described in section 2.4 were chosen as the optimum performance of LC-MS/MS which was usually used for carbofuran analysis.

4. Conclusion
Trace determination of carbofuran in water sample was achieved by using LC-MS/MS. Extraction and purification of carbofuran with SPE-C18 was very fast and simple. Recovery with spike technique of 2 and 5 ng/g was 83.87 and 115.88%, respectively and assuming as good result. Result for precision were of 6.28 and 0.47%, indicating good repeatability. The method was sensitive enough to detect carbofuran. Application of these analytical methods to water samples from Cikapundung river was found of carbofuran at 0.2098 ng/g, that was safe as a source for drinking water.
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