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Development of Hollow Fiber Liquid Phase Microextraction Method for Determination of Diazinon Residues in Vegetable Samples

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Abstract

An extraction method based on a combination of hollow fiber liquid-phase microextraction with a high-performance liquid chromatography diode arrendetector (HF-LPME HPLC-DAD) has been developed and demonstrated to analyze pesticide residues in vegetables. This study aims to determine the optimum extraction conditions and validation performance of this method. Diazinon pesticide was selected as the target model analyte. HF-LPME is performed by stacking microliter organic solvent droplets through an HPLC syringe coated with polypropylene hollow fiber by directly dipping it into the sample solution and stirring it during the extraction process. Finally, the organic solvent was put into an HPLC syringe at the end of the extraction. Then, it was injected into the HPLC-DAD at the Avelength of 247 nm. Several important extraction parameters have been optimized. The optimization results showed the type of organic solvent of n-hexane, the length of the hollow fiber of 1.5 cm, the vo 5 ne of the sample solution of 20 mL, and the stirring speed of 600 rpm. The validation performance obtained a limit of detection (LoD) of 0.10 mgL⁻¹, limit of quantification (LoQ) of 0.33 mgL⁻¹, percent recoveries of 99.88%, a coefficient of variation of 3% (n=15), and the enrichment factor of 19,982 times. Under optimal conditions, the developed method was applied to extract diazinon in vegetable matrix samples using the spiking method. Mustard green was selected as a model matrix sample. From the research, the percentage recoveries of diazinon obtained in the mustard green matrix sample are 98.80% -100.41%.

Keywords: Diazinon, hollow fiber liquid-phase microextraction, high-performance liquid chromatography, pesticide residue, vegetable

Introduction

Organophosphate pesticides are a group of chemical compounds widely used in agriculture to prevent agricultural products from being attacked by pests (Pundir et al., 2019). Although the pesticides used have many advantages, these pesticides residues are very harmful to the environment and are toxic to human health (Menezes et al., 2016). Some health problems caused by exposure to pesticide residues include pancreatic disorders, tumors, seizures, and death (Cai et al., 2016; Jokanović, 2018). Based on these negative impacts, the European Union has set guidelines for the maximum permissible level of organophosphate residue in vegetables, fruits, and meats, 20-500 ngL⁻¹ (Hasan et al., 2017). There are several compounds derived from organophosphate pesticides, one of which is diazinon. According to the Ministry of Health and the Ministry of the Agriculture Republic of Indonesia, the maximum residue limit (MRL) for diazinon in vegetables is 0.5

mgL⁻¹. However, in several agricultural products, including meat, beef liver, and beef fat, in Bogor and Lampung, diazinone residues exceeded the permissible BMR (0,9 mgL⁻¹) (Indraningsih & Sani, 2004). It may be due to the limitation of the instrument detector because the analysis of pesticide residues is very challenging due to trace concentration and difficulties in isolating analytes from complex samples. Therefore, the development of a sensitive and selective analytical method to preconcentrate and enhance the instrument's signal is necessary.

The sample preparation step plays an essential role in the analytical method before instrumental analysis. This step aims to clean, concentrate, and isolate the analyte from the complex matrix. However, this step is very complicated and takes a much longer time (Kamaruzaman et al., 2017). Therefore, a method of determining the trace concentration of pesticide residues that is fast, easy, inexpensive, and effective is urgently needed.

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Miniaturization and simplification of analytical methods for convenience and pre-concentration are popular trends in analytical chemistry (Rozaini et al., 2019). It also supports green analytical chemistry by minimizing the small volume of organic solvents during the sample preparation step (Rozaini et al., 2017 7 Several pre-concentration methods based on the liquid-phase microextraction technique have been developed to analyze organophosphate residues, including single drop microextraction (SDME) 6 ang et al., 2018). This method uses microliters of organic solvent as the acceptor phase. Drops of organic solvent hang up on the tip of the microsyringe needle. However, it has a lot of problems, like the unstable droplet that hangs from the stirrer during the process. Therefore, the droplets are protected by using hollow fibers to stabilize them droplets to overcome these drawbacks. The hollow fiber also acts as a filter for the separation of analytes in complex matrices. This liquid extraction method is hollow fiber liquidphase microextraction (HF-LPME) (Cai et al., 2016). HF-LPME has also been successfully applied to extract carbamate pesticide residues (Ma et al., 2014).

5 In this research, the performance of HF-LPME combined with high-performance liquid chromatography was determined to detect diazinon in mustard green. Several critical parameters such as the type of organic solvent, the length of the hollow fiber, the sample volumes, and the stirring speed were optimized. Meth 7 validation parameters were evaluated, including a limit of detection (LOD), the limit of quantifications (LOQ), % recoveries, decisions, and enrichment factors.

Methods

The materials used in this study are HPLC grade Acetonitrile was obtained from *Merck* (Germany). Organic solvents (methanol, toluene, nhexane, and carbon tetrachloride), p.a were purchased from *Sigma Aldrich* (Singapore). Doubledistilled deionized water of at least 18 M Ω was purified by a nano ultrapure water system (Barnstead, USA). Diazinon 250 mg (98,5%) was purchased from *Sigma Aldrich* (Singapore) as the selected pesticide. A standard stock solution of diazinon (1000 mgL⁻¹) was prepare an methanol and stored in a freezer at about 4 °C. Polypropylene hollow fiber membrane (600 µm id, 200 µm wall hickness, and 0,2 µm pore size) was purchased from Membrane (*Wuppertal*, Germany). The mustard green sample was obtained from agricultural land in Sidoarjo, East Java.

HPLC conditions

The HPLC system used, Agilent type 1100 (United Kingdom), diode array detector, and consists of a pump. The column used a Bondapack C-18 reverse phase microcolumn, particle size 10 μ m, column size 3.9 × 300 mm, and column temperature 30 °C. ACN-water mixture of 70:30 (v/v) was used as the mobile phase at a flow rate of 0.8 mL/min under an isocratic pump. The standard solution and the prepared sample were injected in triplicate into the HPLC/DAD instrument and detected at 247 nm. The magnetic stirrer was obtained from Daihan Labtech LMS-1003, with a magnetic stirrer (*Snipbar*) size 12 × 5 mm. Hamilton syringe for HPLC 25 μ L was received from Switzerland.

Hollow fiber liquid-phase microextraction

The setup of the HF-LPME procedure is shown in **Figure 1**. Several important extraction parameters, such as the types of organic solvent, the hollow fiber lengths, the sample volume, and the stirring speeds, were optimized. Under optimal extraction conditions, this developed method was applied to extract diazinon in mustard green matrix samples.



Figure 1. Setup of HF-LPME

The polypropylene hollow for membrane is cut in various sizes (e.g., 1 cm) and sealed at one edge with a sealer machine. The hollow fibers were cleaned with acetone before application in the HP-LPME to remove some contaminants. A Hamilton microsyringe 25 µL for HPLC was used to transfer the acceptor phase into the sample solution. A microsyringe neglile containing an acceptor phase (e.g., n-hexane 3μ L) was inserted into the rubber septum of the sample vial. The tip of the microsyringe needle is then inserted into the hollow ther segment. The hollow fiber is immersed in the acceptor phase (e.g., n-hexane) for 5 s to impregnate the pores of the hold w fiber before being inserted into the needle tip. The assembly was immersed in the sample solution (a standard solution of 6 ppm diazinon was used). The extraction process begins by stirring the sample solution for a specific time (e.g., 10 min) u10g a magnetic stirrer. Finally, the extraction, the acceptor phase was inserted into a microsyringe and then directly injected into the HPLC-DA5 at a wavelength of 247 nm. Several important extraction parameters such as the type of the organic solvent (toluene, n-hexane, and carbon tetrachloride), the lengths of the hollow fiber (1, 1.5, 2, and 2.5 cm), the volume of the sample solutions (15, 17.5, 20, 22.5, and 25 min), and the stirring speeds (300, 400, 500, 600, and 700 ppm) have been optimized. The other variables are kept constant in optimizing one of the extraction

parameters. For example, optimization of the types of organic solvents, other variables were held regular (length of hollow fiber of 2 cm, the volume of organic solvent of 3 μ L, stirring speed of 500 rpm, extraction time of 15 minutes, and volume of standard diazinon solution (6 ppm) of 20 mL). The optimum extraction conditions were evaluated and applied to extract diazinone in vegetable matrix samples. Green mustard was chosen as vegetable matrix samples to assess the performance of the proposed method in the actual sample condition.

Results and Discussion

Peaks separation

Diazinon analysis was carried out at optimum separation and quantification conditions in HPLC-DAD. The best conditions were found at 247 nm when the flow rate was 0.8 mL/min, and the mixture of ACN and water was 70:30 (v/v). The reversed phase column C-18 was chosen to separate diazinon. For non-polar analytes, like diazinon (Log Kow = 3.81), this column is the best choice for this type of test. Not only the kind of column but the composition of the mobile phase also plays a vital role during the separation process. ACN-water mixture 70:30 (v/v) showed a good separation peak for diazinon analysis. The separation of the diazinon peak in the solvent n-hexane is illustrated in **Figure** 2.



Figure 2. Chromatogram of sample separation: 1. acetonitrile, 2. methanol, 3. n-hexane, 4. diazinone (6 ppm)

Optimization of the types of organic solvent

There were three kinds of organic solvents optimized as extractor phase for extraction of diazinons, such as toluene, n-hexane, and carbon tetrachloride. Each type of organic solvent was replicated 3 times. HPLC-DAD then analyzed the extracted solution. The organic solvent n-hexane showed optimal results. n-hexane has the lowest solubility in water at 25 °C (9.5 mg/mL) compared to toluene and carbon tetrachloride. It causes the extracted diazinon in n-hexane to be not quickly released back to the donor phase. In addition, based on the Log Kow value of diazinone (3.81), it is closest to the Log Kow of n-hexane (3.9) compared to the Log Kow of toluene (2.73) and carbon tetrachloride (2.04), this causes diazinone to be easier to extract in n-hexane. The most important thing to keep in mind when choosing organic solvents is that the organic solvent should be able to stick to the hollow fibers, dissolve well with analytes, become insoluble in water, and not evaporate quickly during extraction (Esrafili et al., 2018). Then, n-hexane is used for more parameter optimization. **Figure 3** shows the types of organic solvent optimization that worked best.



Figure 3. Optimization of the type of organic solvents

Optimization of the length of the hollow fiber

The optimization of the length of the hollow fiber is done by varying the length by 1; 1.5; 2; and 2.5 cm. Each variation of perforated fiber length was repeated 3 times. HPLC-DAD then analyzed the extracted solution. The optimum hollow fiber length for flazinon extraction using the HF-LPME method is 1.5 cm. The shorter the hollow fiber, the less spread of the acceptor phase on the surface of the hollow foer, so that the diazinon that enters through the pores of the hollow fiber is also less and produces a smaller peak area. The longer the hollow fiber, the more phase acceptor (organic solvent) dissociated on the surface of the hollow fiber so that more diazinon is extracted. The length of the perforated fiber and the volume capacity of the perforated fiber is regulated by the size of the bottle used for extraction (Gjelstad, 2019; Mlunguza et al., 2020). The more extended length of the hollow fiber, the more organic solvents will spread to the pores of the hollow fiber, but at the time of extraction, it cannot be reached by the microsyringe needle. For that, it causes the extracted diazinon to be smaller. The hollow fiber length of 1.5 cm was used to optimize the analytical parameters further. At the size of the hollow fiber is more than 2.5 cm, the hollow fiber will contact with magnetic stirrer so that it will be disturbed by the kinetic force and separated from the microsyringe needle. The optimization of the length of the hollow fiber is shown in Figure 4.



Figure 4. Optimization of the length of the hollow fiber

Optimization of the sample solution volume

By adjusting the sample volume to 15, 17.5, 20, 22.5, and 25 mL, the sample solution volume is optimized. Each modification in the volume of the sample solution was repeated three times. The extracted solution was then evaluated using HPLC-DAD. The optimal volume of sample solution for diazinon extraction using the HF-LPME method is 20 mL. The larger volume of the sample solution contains more analytes (Salvatierra-stamp et al., 11 8; Venson et al., 2019). Combining a small volume of acceptor solution and a large volume of factor (Raharjo et al., 2009; Salvatierra-stamp et al., 2018). The sample solution volume of 20 mL was used to optimize the analytical parameters further. The

results of the optimization of the sample volume are shown in **Figure 5**.

Optimization of the stirring speed

The optimization of stirring speed was carried out with variations of 300, 400, 500, 600, and 700 rpm. Each variation of mixing speed was repeated 3 times. HPLC-DAD then analyzed the extracted solution. Stirring can increase the mass transfer rate during the extraction process (Zuluaga et al., 2021). Stirring the sample solution with the HF-LPME method can shorten the time to reach thermodynamic equilibrium, especially for analytes with a larger molecular mass. In the HF-LPME method, the organic solvent is covered and protected by a hydrophobic hollow fiber, making it easier to control the higher stirring speed (Zuluaga et al., 2021).





Figure 5. Optimization of the sample volume

Based on Figure 5, it can be seen that the optimal stirring speed is 600 rpm. The higher the stirring speed, the higher the transfer rate of the analyte to the organic solvent. However, a higher stirring speed can result in the release of the extracted diazinon on the surface of the hollow fiber to be released back into the sample solution (Cai et **7**, 2016). The increasing stirring speed can generate bubbles of air within the pores of the fiber, making it difficult mass transfer from the donor

phase to the lumen of the thread (Zuluaga et al., 2021). Higher stirring speed also increases the kinetic force so that it will disturb the position of the hollow fiber immersed vertically in the sample solution. The stirring speed of 600 rpm is the optimal result for diazinon extraction by the HF-LPME method and is used for standard curve extraction. The optimization of the stirring speed results is shown in Figure 6.



Figure 6. Optimization of the stirring speed

Validation method

After obtaining optimal conditions, several standard solution concentrations were prepared in deionized water. The stock solutions were treated using the HF-LPME method and directly injected into the HPLC-DAD. Extraction replication using the HF-LPME method at each concentration of the standard solution was carried out three times. Linearity range, detection limit (LoD), quantification limit (LoQ), % recovery (%R), precision or coefficient of variation (% CV), and enrichment factor (EF) were evaluated.

A good linearity range was achieved at 0.200-10.000 mgL⁻¹ with a coef 3 ient correlation (r^2) of 0.9999, which indicated that the analyte showed good linearity in each concentration range. The LoD and LoQ values of the developed method are 0.10 mgL⁻¹ and 0.33 mgL⁻¹, respectively. The proposed method offers excellent repeatability with %CV less than 3%. Meanwhile, the relative recovery was 99.88%. The detailed results are presented in Table 1.

The enrichment factor (EF) plays a vital role in the microextraction technique. The enrichment factor described the pre-concent 10 on phenomenon that occurs by the distribution of the analyte from the sample solution (V_{aq}) to the acceptor phase (V_{org}) in a smaller volume. It is also closely related to the distribution constant as a fundamental principle in liquid-liquid extraction. Determination of EF can be defined by equations (1), (2), and (3) (Raharjo et al., 2009; Zuluaga et al., 2021).

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I able 1. Analytical performance of HF-LPME HPLC-DAD							
	Recovery	Enrichment	Linearity	Correlation	LoD	LoO	Coefficient
Analyte	raccovery	Factor	range	coefficient	LOD	202	of variation
	(%)	(times)	(mgL ⁻¹)	(r ²)	(mgL^{-1})	(mgL ⁻¹)	(%)
Diazinon	99.88	19,982	0.200- 10.000	0.9999	0.10	0.33	3.00

$$EE = \frac{n_{org}}{n_o} , EE = \frac{K_{org/aq} V_{org}}{K_{org/aq} V_{org} + V_{aq}}$$
(1)

$$EE = \frac{C_{org}}{C_o}, EF = \frac{n_{org} V_{aq}}{n_o V_{org}}$$
(2)

$$EF = \frac{EE \cdot V_{aq}}{V_{org}} \tag{3}$$

where EE is the expaction efficiency and EF is the enrichment factor. C_{org} and n_{org} are the concentration and mass of the extracted analyte in the organic solvents, respectively. C_o and n_o are the concentration and mass of the analyte initially present in the sample solution, respectively. V_{aq} and

 V_{org} are the volume of the sample solution and the volume of the acceptor phase, respectively.

Application of the proposed method in a. vegetable matrix sample

The vegetable sample matrix was prepared by diluting 10 gr of crushed mustard green in 100 mL of deionized water. The solution was then allowed to stand for 3 hours. The filtrate and sedimentation were separated using a Buchner filter. The filtrate was treated using the HF-LPME method under optimal extraction conditions and directly injected into the HPLC-DAD. Each of the three different standard solution concentrations was added to the sample matrix by spiking. This way, we could see how much of the sample was recovered. The performance of the proposed method in the vegetable matrix sample is presented in Table 2.

Table 2. Recoveries of diazinon in vegetable sample matrix using HF-LPME HPLC-DAD

Analyta	Recovery on spiking method (%)				
Analyte	0 ppm	2 ppm	6 ppm	10 ppm	
Diazinon	ND	98.90	100.41	99.63	

The analyte recovery is excellent, with 9 value greater than 98.90%. The results showed that the proposed method was suitable for the determination of trace amounts of pesticide residues in vegetables, especially diazinon.

Comparison of the proposed method with Ь. other previous methods for determination diazinon residue

The comparison of HF-LPME-HPLC-DAD with other reported methods for the analysis of diazinon residue in the vegetable appeared in Table 3.

Table 3. Comparison of the proposed method with other published methods for the determination of dianin

ulazilioli					
Extraction technique-	Sample	%R	LoQ	Ref.	
Instrument	-				
DSPE-GC-NPD	Potato	95.76-	0.06 mgkg ⁻¹	(Saraji et al., 2021)	
		99.87			
LLE-GC-FID	Lavender and	100.90	0.085 mgL ⁻¹	(Rezk et al., 2018)	
	Rosemary leaves		-		
HF-LPME-HPC-DAD	Green Mustard	99.88	0.33 mgL ⁻¹	Present work	

Generally, each published method has advantages and disadvantages. Dispersive solidphase extraction (DSPE) method combination with gas chromatography-nitrogen phosphorous detector (GC-NPD) for analysis diazinon in potato needs solid sorbent. Therefore, to minimize carryover analyte in the porous sorbent need more treatment and time to desorb the analyte completely (Saraji et al., 2021). While liquid extraction (LLE) method, combination with gas chromatographyflame ionization detector (GC-FID), needs a larger volume of organic solvent, so it produces more organic waste (Rezk et al., 2018). LoQ in this work is different from other methods because of the instrument detector. However, another validation parameter of the present work with the previous method is not significantly different, so it can be used as a simple extraction method to remove diazinon residue from a vegetable sample.

Conclusions

The HF-LPME combined with HPLC-DAD provides good performance for determining diazinon pesticides in vegetable matrix samples. High percent recovery and extraction efficiency are achieved. The low value of LoD, LoQ, and %CV indicates that the proposed method has excellent potential for residue pesticide analysis.

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