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Application of High Performance Liquid Chromatography Method for Triadimenol Analysis in Water Sample

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Abstract. Triadimenol is the most important triazole fungicide used for prevention and treatment of plant fungal disease. A simple high performance liquid chromatography (HPLC) method for triadimenol analysis in water sample is reported in this study. The optimized HPLC system using C₁₈ column was achieved using mobile phase composition containing acetonitrile-water (70:30, v/v), a flow rate of 1.0 mL/min, UV detection at 220 nm, and analysis time within 2.5 min. The calibration graph was linear in the range 5 to 50 mg/L with r^2 of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 1.23 and 4.12 mg/L, respectively. Combination of solid phase extraction (SPE) and the optimized HPLC method has been successfully applied for the determination of triadimenol in water sample with a recovery of 94.24 % (RSD = 5.38%, $n = 4$). The proposed HPLC method provides short analysis time, high reproducibility and high sensitivity.

Keywords: Fungicides, HPLC, SPE, Triadimenol, Water sample.

1. Introduction

The major categories of pesticides classified by target are insecticides, herbicides, and fungicides. Fungicides are used to eradicate or prevent the undesirable growth of fungal microorganisms in many agricultural, horticultural, and industrial situations. Numerous substances possess antifungal activity, and their chemical structural spectrum is wide and diverse, covering both organic and inorganic substances. The most important category of fungicides is triazole-type because of their excellent protective and curative [\[12\]](#)ver against a wide spectrum of crop disease. Triadimenol {1-(4-chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazole-1-yl}butan-2-ol}, one [of the](#) triazole fungicide, is a systemic fungicide for used protective, curative, and irritative. It is recommended for use as either fungicides or plant growth regulators [\[1-3\]](#).

Chromatographic methods used so far for the analysis of triadimenol include gas chromatography (GC) [\[4\]](#), supercritical fluid chromatography (SFC) [\[5\]](#), gel permeation chromatography and ultra performance convergence chromatography/tandem mass spectrometry [\[6\]](#). High performance liquid chromatography (HPLC) method has also been used for triadimenol analysis. The advantages of HPLC method are rapid analysis, required small sample volume, column can be used many times, and high level of selectivity, sensitivity and accuracy [\[7-9\]](#).



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Combination of solid phase extraction (SPE) and the HPLC method has been successfully applied in this study for the determination of triadimenol in river water sample. The HPLC method using C₁₈ column and UV detection was optimized in this study. Structure of triadimenol is shown in figure 1.

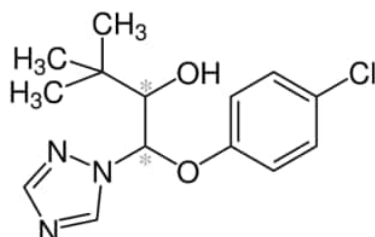


Figure 1. Chemical structure of triadimenol

2. Methods

2.1. Materials

Triadimenol was obtained from Riedel-de Haen (Seelze, Germany). All other chemicals and solvents were common brands of analytical reagent grade or better and were used as received. Deionized water was collected from a Millipore water purification system (Molsheim, France). All solvent were degassed prior to usage. River water sample was obtained from local river in Purwokerto, Indonesia. The stock solution of triadimenol (1000 mg/L) was prepared by dissolving triadimenol standard in methanol. A series of standard solutions (5 to 50 mg/L) were prepared by diluting the stock solution with methanol. The stock and all standard solutions were labeled and sealed with wrapping to avoid evaporation, and were stored in the refrigerator prior to use.

2.2. Instrumentation

The HPLC system with UV-Vis detection (Hitachi L-2000 series, Japan), equipped with a model L-2130 pump, an on-line solvent vacuum degasser, an autosampler with 10 μ L injector loop and a UV-Vis detector L-2420 at 220 nm. The separation was carried out in a Perkin Elmer C₁₈ μ m 150 x 4,6 mm. The mobile phase consisted of acetonitrile-water (v/v) and operated isocratically.

2.3. HPLC optimization

Triadimenol standard solution 50 mg/L was analyzed using the HPLC system with various of flow rates (0.8; 0.9; and 1.0 mL/min), and various of mobile phase acetonitrile-water compositions (70:30, 60:40, 50:50 v/v).

2.4. Solid phase extraction (SPE) procedure

A representative portion of water samples (river water sample and spiked river water sample containing triadimenol standard of 5 mg/L) were passed under vacuum through a C₁₈ solid-phase column (Supelclean SPE T₁₈s from Supelco), which was preconditioned with 5 mL of methanol and 5 mL of deionized water. Analytes were then eluted with 3 mL of methanol. The eluate was then diluted with methanol (total volume of 5 mL) before injected into the HPLC system,

2.5. Analytical performance of the HPLC method

The performance of the method was examined in terms of the linearity, repeatability, LOD and LOQ. Linearity of the HPLC method was assessed by constructing the calibration curves of peak areas against the concentration of standards (5 – 50 mg/L). The repeatability was determined in four replicates at triadimenol concentration of 50 mg/L. The limit of detection (LOD) and limit of

quantification (LOQ) were determined by the calibration curve along with the signal-to-noise ratio (S/N) as 3 and 10, respectively.

3. Results and Discussion

3.1. Optimization of flow rate

The effect of different flow rate was first investigated in this study. Flow rate was varied in three different flow rates i.e. 0.8; 0.9; and 1.0 mL/min. The typical result of HPLC chromatogram of triadimenol with a different flow rate of the mobile phase is shown in figure 2. It can be seen that the retention time of triadimenol was gradually increased when the flow rate of mobile phase was decreased. The 1.0 mL/min has been chosen as the optimized flow rate because it gives the best baseline of triadimenol peak with retention time less than 2.5 min.

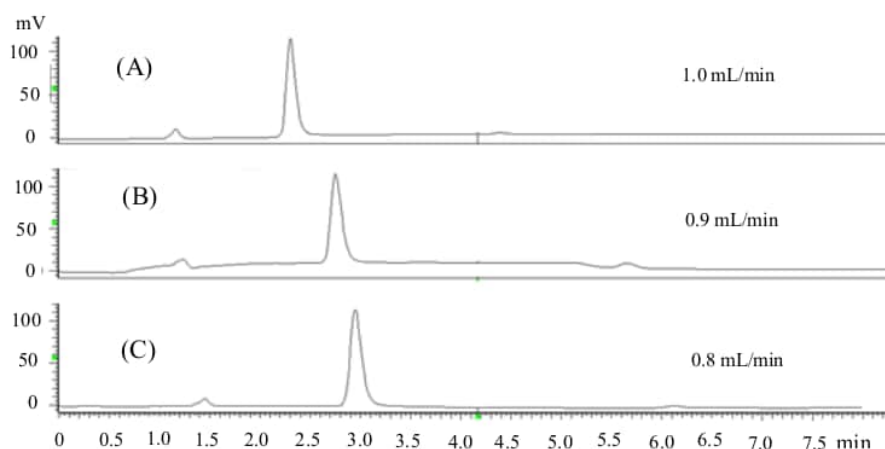


Figure 2. Chromatogram of triadimenol (50 mg/L in methanol) by HPLC with different flow rate conditions (A) 1.0 mL/min, (B) 0.9 mL/min, (C) 0.8 mL/min, mobile phase composition acetonitrile-water (60:40, v/v); injection volume 10 μ L.

3.2. Optimization of mobile phase

The effect of different mobile phase was then investigated in this study. The mobile phase varied was in three different percentages of acetonitrile-water (v/v) such as (50:50); (60:40); and (70:30). The typical result of the HPLC chromatogram of triadimenol with a different mobile phase is shown in figure 3. Addition of water reduce the elution strength of the mobile phase, therefore the analyte was eluted slower. The acetonitrile-water (70:30, v/v) has been chosen as the optimized mobile phase because it gives the best baseline of triadimenol peak with retention time less than 2.5 min.

3.3. Analytical performance of the HPLC method

The performance of the developed HPLC method was examined in terms of linearity, limit of detection (LOD), limit of quantification (LOQ) and repeatability. Calibration curve was linear over the

concentration range of 5 - 50 mg/L of the triadimenol standard with $r^2 = 0.9997$. The LOD and LOQ of triadimenol were 1.23 and 4.12 mg/L, respectively. The low LOD and LOQ showed that the developed HPLC method is sensitive and sufficient to determine triadimenol in water samples for routine analysis. The optimized HPLC method combined with solid phase extraction (SPE) pretreatment by using C_{18} solid-phase column is applicable for determination of triadimenol in the river water sample (figure 4). The percentage recovery obtained for analysis of triadimenol in water sample was 94.24 % with RSD of 5.41% ($n = 4$).

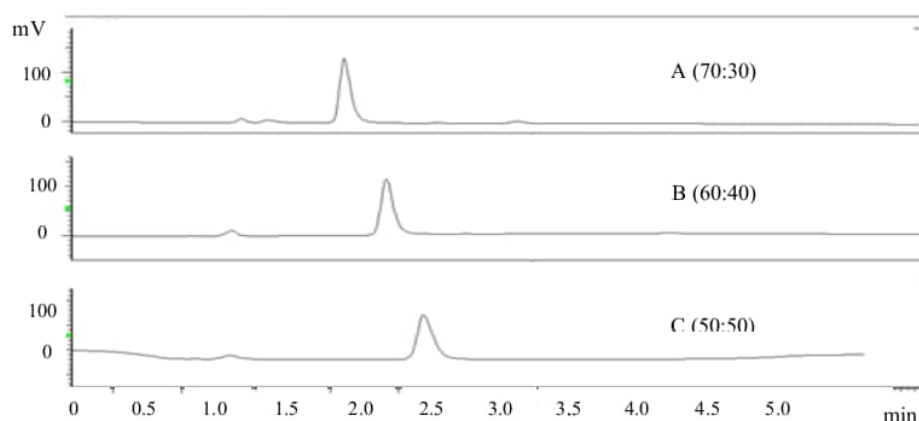


Figure 3. Chromatogram of triadimenol (50 mg/L in methanol) by HPLC with different mobile phase composition (A) acetonitrile-water (70:30, v/v), (B) acetonitrile-water (60:40, v/v), (C) acetonitrile-water (50:50, v/v); flow rate condition 1.0 mL/min; injection volume 10 μ L.

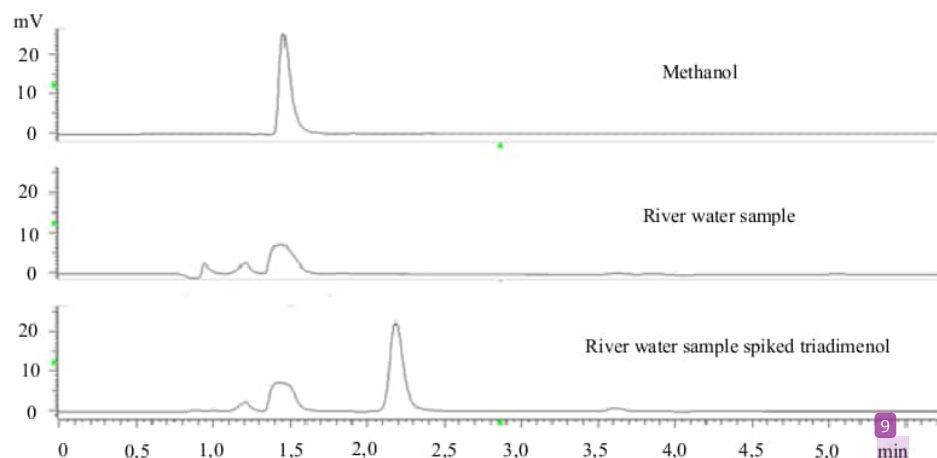


Figure 4. Chromatogram of solvent (methanol), river water sample and spiked water sample by triadimenol standard (5 mg/L), respectively SPE-HPLC with UV detector (220 nm), mobile phase composition acetonitrile-water (70:30, v/v), flow rate condition 1.0 mL/min, injection volume 10 μ L.

4. Conclusions

In the present work, the high performance liquid chromatography (HPLC) method using C₁₈ column was successfully developed for the determination of triadimenol in the river water sample. The optimized HPLC condition was achieved using mobile phase composition containing acetonitrile-water (70:30, v/v), the flow rate of 1.0 mL/min, and UV detection at 220 nm. The calibration graph was linear in the range from 5 to 50 mg/L with r^2 of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 1.23 and 4.12 mg/L, respectively. The optimized SPE-HPLC method was successfully applied to the analysis of triadimenol in the river water sample. The percentage recovery obtained for analysis of triadimenol in water sample was 94.24 % with RSD of 5.41% ($n = 4$).

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