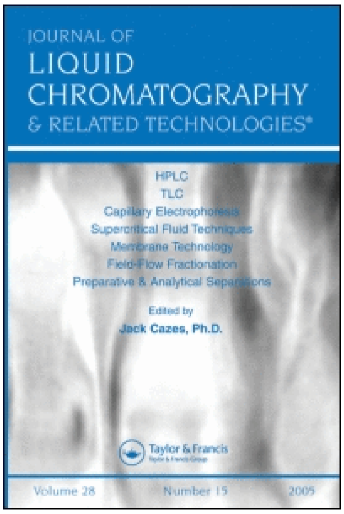

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Determination of Tricyclazole in Water Using Solid Phase Extraction and Liquid Chromatography

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Abstract: A method for determination of tricyclazole in water using solid phase extraction and high performance liquid chromatography (HPLC) with UV detection at 230 nm and a mobile phase of acetonitrile:water (20:80, v/v) was developed. A performance comparison between two types of solid phase sorbents, the C_{18} sorbent of Supelclean ENVI-18 cartridge and the styrene-divinyl benzene copolymer sorbent of Sep-Pak PS2-Plus cartridge was conducted. The Sep-Pak PS2-Plus cartridges were found more suitable for extracting tricyclazole from water samples than the Supelclean ENVI-18 cartridges. For this cartridge, both methanol and ethyl acetate produced good results. The method was validated with good linearity and with a limit of detection of $0.008\,\mu g\,L^{-1}$ for a 500-fold concentration through the SPE procedure. The recoveries of the method were stable at ~80% and the precision was from 1.1 – 6.0% within the range of fortified concentrations. The validated method was also applied to measure the concentrations of tricyclazole in real paddy water.

Keywords: C_{18} sorbent, HPLC, Paddy water, Solid phase extraction, Styrene-divinyl benzene copolymer sorbent, Tricyclazole

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INTRODUCTION

In recent years, the public concern about the risks arising from the intense use of pesticides in agriculture has become more imminent. Pesticide pollution is the main environmental issue for regions close to the rice growing areas, since pesticides in the rice field are easily discharged to the open environment. Therefore, more stringent regulations on pesticide residue in water were issued by authorities around the world. For example, the European Union has issued the Directive 98/83,[1] concerning the quality of water designated for human consumption, and established the maximum tolerable concentration of one individual pesticide at 0.1 $\mu g\,L^{-1}$, and the total amount of pesticides at 0.5 $\mu g\,L^{-1}$ (including their main metabolites). To achieve this regulation, an analytical method should be simple and reliable for routine monitoring programs.

Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b]benzothiazole) (Figure 1), a systemic fungicide, is commonly used to control the popular rice blast disease, especially in Asia. In some countries, like Portugal, tricyclazole is the only fungicide registered for rice blast.[2] The potential environmental risk of tricyclazole is considered significant because it is relatively stable in water-soil systems.[3] Consequently, a method to measure the environmental concentrations of tricyclazole is needed to assess its risk in the aquatic environment.

Current methods for measuring tricyclazole concentration used the liquid–liquid extraction method.[3–5] This extraction method was considered less environmental friendly than the solid phase extraction (SPE), which also has been used to extract pesticides in water samples, because the SPE method uses much less solvents than the liquid–liquid extraction method. Other advantages can be obtained from the use of the SPE method including higher effectiveness for polar compounds, higher sample throughput, and lower cost.[6] It is thus of high interest to apply the SPE method for tricyclazole. However, most of the available SPE methods are designed using the popular C_{18} sorbent. Meanwhile, more and more studies have introduced the great potential of styrene-divinyl benzene copolymer sorbent for pesticide analysis, especially for polar compounds like tricyclazole.[6]

Besides using the liquid–liquid extraction method, the above mentioned studies on tricyclazole fate and transport in the environment

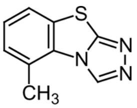

Figure 1. Structure of tricyclazole.

also utilized the gas chromatographic (GC) technique.[3–5] However, liquid chromatography (LC) is favored over GC for low volatile and polar pesticides such as tricyclazole. Since the majority of pesticides strongly absorb in the UV region, diode array detection (DAD) with LC is a good option for detection of pesticides, because this also permits a confirmation of peak identity, utilizing the UV spectrum.[7]

Therefore, the aim of this study is to compare the performance of these two sorbents in extracting tricyclazole from water and then to validate a HPLC method using the appropriate SPE sorbent for measuring tricyclazole concentration in water.

EXPERIMENTAL

Chemicals and Reagents

Tricyclazole (analytical grade, >98.8%) and solvents of analytical grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was produced with a MilliQ Water Purification System (Millipore, Billerica, MA, USA). The solid phase extraction cartridges for the comparison were Supelclean ENVI-18 (Supelco, Bellefonte, PA, USA), which is packed with C_{18} sorbent (size 6 mL, 1 g) and Sep-Pak PS2-Plus (Waters, Milford, MA, USA), which is packed with styrene-divinyl benzene copolymer sorbent (short body, 300 mg).

Instrumentation

All samples were analyzed by the Waters Alliance HPLC system consisting of the 2695 Separations Module and the 2996 diode array detector controlled by the MassLynx software from the computer. The analytical column was a Wakopak Wakosil C_{18} (250 × 4.6 mm, 5 µm particle size), which was kept at 40°C during the analytical run. The detection was performed at 230 nm. The pump was set in isocratic mode at the rate of $1\,\mathrm{mL\,min^{-1}}$ with the mobile phase of acetonitrile:water (20:80, v/v). Sample injection volume was 20 µL.

For the analysis, the stock standard solution ($200\,\mathrm{mg\,L^{-1}}$) was prepared by dissolving 10 mg of tricyclazole in a 50 mL volumetric flask with pure methanol. This stock standard solution was then diluted with methanol to obtain a working standard solution ($10\,\mathrm{mg\,L^{-1}}$). Analytical standards (0.1, 0.5, 1, 5, and $10\,\mathrm{mg\,L^{-1}}$) for HPLC calibration were prepared from aliquots of the working standard solution by evaporating the solvent to dryness and dissolving the residue in acetonitrile:water (20:80, v/v).

Solid Phase Extraction

Both types of cartridges, Supelclean ENVI-18 and Sep-Pak PS2-Plus, were preconditioned with 5 mL of methanol and 5 mL of water. Blank and spiked water samples were then passed through the cartridge with the rate of 10 mL min^{-1}, which was reported as most appropriate for the SPE cartridge to achieve high recovery.[8] Care was taken to avoid drying the cartridge. After this enrichment procedure, the cartridges were washed with 10 mL of water and then were dried by pulling the air through during about 20 min before eluting adsorbed tricyclazole with 5 mL of methanol or ethyl acetate, at the rate of 1 mL min^{-1}. The extracts were collected and evaporated to 1 mL by a rotory evaporator and to dryness by a gentle stream of nitrogen. The residues were redissolved in 1 mL of acetonitrile-water (20:80, v/v) for HPLC analysis.

Sample Preparation

Deionized water was used as a blank sample. Spiked samples were prepared by spiking deionized water with the working standard solution of tricyclazole (10 mg L^{-1}) to obtain the final concentrations of 0.05, 0.1, 0.2, 0.4, and 1.0 μg L^{-1}. The water samples were then extracted by SPE before HPLC analysis. Four replicates of each spiked level were extracted and analyzed. The results were subjected to statistical analysis using GraphPad Prism 5 software (GraphPad Software Inc., CA, USA).

The real samples were taken from 2 paddy plots, which had been applied with tricyclazole in the experimental farm of Tokyo University of Agriculture and Technology, Tokyo, Japan, at different intervals after pesticide application. The samples were subjected to one of the SPE procedures after the comparison is made and then were analyzed by HPLC.

RESULTS AND DISCUSSION

This reversed phase HPLC method, with DAD, has proven to be a good alternative for tricyclazole determination because it has a shorter retention time of 6.1 min compared to the retention time of 16.4 min,[3] or more than 40 min[5] of the GC-NPD method. The isocratic mobile phase acetonitrile-water (20:80, v/v) produced good a chromatogram without the necessity of adjusting its pH, as in another study for the polar pesticide bentazone.[9]

Linearity of Calibration Curve

Linearity of the calibration curve is important and should be evaluated across the intended range of the analytical procedure.[10] For monitoring purposes, the analytical range should extend to the lowest level of concentration possible of the analyte. Calibration curves of tricyclazole were constructed by plotting concentrations vs peak areas, with 5 concentration levels within the range of $0.1 - 10\,\mathrm{mg\,L^{-1}}$. Linearity was evaluated both by visual observation and linear regression. With three replications, the regressed equation in this concentration range was $y = (2006 \pm 2.830)x + (17.09 \pm 20.44)$. The correlation coefficient was 1.

Stability of Tricyclazole Standard

The stability of the tricyclazole standard solutions should be investigated to confirm the robustness of the method.[10] The standard solutions of tricyclazole used to construct the calibration curves were kept at 4°C and reanalyzed after 90 days. The regressed equation of the calibration curve produced by the stored standard solutions was $y = 2015.2x - 97.53$ with $R^2 = 0.9999$ and therefore, was not significantly different from those produced by the fresh solutions.

Solid Phase Extraction

The silica based C_{18} sorbent is a very popular SPE sorbent but it has some limitations for polar analytes. This problem seems to be solved by the carbon based sorbent styrene–divinylbenzene (PS–DVB) copolymers with high specific areas in the range 500 to $1200\,\mathrm{m^2\,g^{-1}}$ and high degree of purity.[6] Supelclean ENVI-18 (C_{18} sorbent) and Sep-Pak PS2-Plus (PS–DVB sorbent) were subjected to the same test to see their performance in extracting tricyclazole from spiked water samples.

For both cartridges, the blank samples did not produce any signal at the retention time region. However, ethyl acetate seems to give less interference than methanol. The use of either methanol or ethyl acetate as eluting solvent would not interfere with the possible peak of the target compound.

Concerning the spiked samples, there was a significant difference between the performances of the two cartridges. The Sep-Pak PS2-Plus cartridge gave higher recovery ratios and lower relative standard deviation of the results (Table 1) whether methanol or ethyl acetate was used. The Supelclean ENVI-18 cartridge produced lower recovery ratios as well as a higher RSD value. It is because tricyclazole is polar with relatively high solubility so C_{18} is not a suitable sorbent. Morrica et al.[11] observed that conventional SPE using reverse phase solid sorbents such as C_{18} is strongly dependent on the polarity of the analyte and it produced less than satisfactory results when it was applied to the extraction of highly

Table 1. Recovery and precision of Sep-Pak PS2-Plus and Supelclean ENVI-18 cartridges

	Recovery (%)		RSD (%)	
Eluting solvent	Sep-Pak PS2-Plus	Supeclean ENVI-18	Sep-Pak PS2-Plus	Supeclean ENVI-18
Methanol	88.4	84.6	1.8	22.2
Ethyl acetate	91.0	53.9	5.8	5.3

polar compounds from a large volume of water, as breakthrough of these analytes occurred. This means that the conventional adsorption mechanism is not applicable to hydrophilic compounds, like tricyclazole. Eventually, the Sep-Pak PS2-Plus cartridge and methanol was used to validate the method. Examples of blank and spiked samples extracted by Sep-Pak PS2-Plus cartridges are shown in Figure 2.

Limit of Detection

The limit of detection was determined using the method specified by Eurachem validation guidelines.[12] This approach measured 10 independent sample blanks fortified at lowest acceptable concentration/lowest calibration concentration to calculate the standard deviation. Limit of detection was 0.008 μg L^{-1} for a 500-fold concentration through SPE. The limit of detection established in this study is comparable to those for other pesticides using SPE methods.[8,9,13] The corresponding limit of quantification was 0.028 μg L^{-1}, which is lower than the EU standard for any pesticide in water of 0.1 μg L^{-1} and thus the method is applicable for routine regulatory purposes concerning tricyclazole in water.

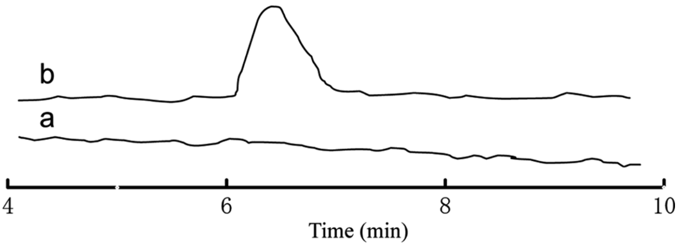

Figure 2. Chromatograms obtained at 230 nm by HPLC with DAD detector. a: extraction from blank sample; b: extraction from sample spiked with tricyclazole at 0.1 μg L^{-1}.

Accuracy and Precision

As the analytical conditions were defined, the method validation was conducted with Sep-Pak PS2-Plus cartridges. The sample volume is important for samples of environmental interest. A sample volume higher than 200 mL is necessary in order to determine low levels of pollutants.[13] In the present work, the sample volume was 500 mL because this volume could help concentrate the sample for the determination of low levels of tricyclazole. The test was carried out by four trials at five fortification levels.

Accuracy and precision are the most important criteria to evaluate the performance of an analytical method. Precision (in-house repeatability) is defined as the closeness of results when repetitive analyses are made on the same sample. Meanwhile, the accuracy is defined as the bias between the arithmetic mean of test results and the true value or reference value. Besides using the standard certified reference materials, the accuracy can also be assessed through the recovery of spiked samples. The results are reported in Table 2.

The recoveries of tricyclazole from water at five fortified concentrations (four replicates) were acceptable and stable over the concentration range (~80%). The repeatability was very good, with RSD of 1.1 – 6.0% for all fortification levels. These results were also satisfactory to the criteria set by the European Union for pesticide residue analysis.[14]

Analysis of Real Samples

The real water samples taken from paddy fields were analyzed by SPE using Sep-Pak PS2-Plus cartridges followed by HPLC with DAD. The concentrations of tricyclazole in 6 samples are shown in Figure 3. The results indicated the dissipation of tricyclazole in the paddy water over time. However, concentrations of tricyclazole in paddy water were still high after several weeks from the application date. Therefore, care should be taken to avoid the discharge of paddy water to the environment.

Table 2. Average recoveries obtained following pre-concentration of 500 mL of water samples spiked with tricyclazole

Spiked concentration	$0.05\,\mu g\,L^{-1}$	$0.1\,\mu g\,L^{-1}$	$0.2\,\mu g\,L^{-1}$	$0.4\,\mu g\,L^{-1}$	$1.0\,\mu g\,L^{-1}$
Recovery (%)	78.5	80.7	80.1	79.6	80.1
RSD (%)	1.5	1.1	4.7	6.0	2.5

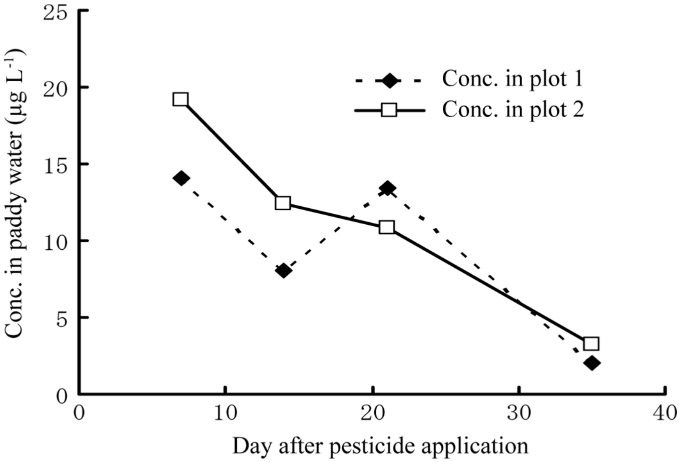

Figure 3. Concentrations of tricyclazole in paddy water from actual paddy plots.

CONCLUSION

The study presented a reproducible and accurate method for analysis of tricyclazole in water samples using solid phase extraction and reversed phase HPLC with DAD at 230 nm. Extraction of tricyclazole in water samples by SPE using the Sep-Pak PS2-Plus (PS–DVB sorbent) cartridge produced better results than the conventional Supelclean ENVI-18 (C_{18} sorbent). The limit of detection and the limit of quantification of the method for tricyclazole in water are low enough to meet the international requirements of 0.1 µg L^{-1}. The method gave good recoveries and repeatability for the spiked water samples analyzed. The major advantage of the method is that less labor and time are required for the analysis.

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