Notes

A Clean-up Method by Photocatalysis for HPLC Analysis of Iprodione in Dry Basil

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Titanium dioxide was used as a photocatalyst to decompose interfering substances for a quantitative analysis of a fungicide (iprodione) in dry basil by HPLC. A quartz vial containing basil extract and titanium dioxide was irradiated with black light. The interfering substances were almost completely decomposed by 180 min of irradiation, whereas 88.3% of iprodione remained. The recovery of iprodione was 102.6% by the proposed method in basil extracts. This may have been due to different decomposition rates of the analyte and interfering substances.

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Introduction

Titanium dioxide is a known photocatalyst, which exhibits oxidative and super-hydrophilic activities under UV irradiation.[1]

The oxidative activity of titanium dioxide has been widely used in various applications, such as the degradation of malodorous substances in air,[2] the sterilization of microorganisms,[3] and drainage treatments.[4] For example, in drainage treatments titanium dioxide is used for the degradation of pollutants. In particular, treatments using titanium dioxide are attracting attention for the decomposition and detoxification of environmental pollutants, such as agricultural chemicals,[5] PCBs,[6] dioxins,[7] and endocrine disruptors.[8–10]

In general, agricultural chemicals in water, food, and soil are analyzed by GC-MS, LC-MS, *etc.*, after being extracted using organic solvents, and purified using solid-phase cartridge columns.[11,12] However, purification using a cartridge column is not sufficient for the complete removal of many interfering substances in extracts of dry fruits, vegetables, processed foods, and other foods. Coexisting substances in foods often complicate the detection of agricultural chemicals by UV-Vis and fluorescence spectroscopy because of interfering peaks, and by LC-MS analysis because of insufficient ionization of the agricultural chemicals. The purification process should be simple and brief, because an elaborate process may consume more time, and it may decrease the recovery rate of the target chemical. From the viewpoint of food safety and hygiene, the establishment of a highly accurate analytical method is required, particularly for agricultural chemicals in foods.

Consequently, we examined the possibility of a new clean-up method using titanium dioxide for the HPLC analysis of agricultural chemicals.

This study examined the effectiveness of titanium dioxide as a photocatalyst in the clean-up of extracts of dry basil for HPLC analysis of iprodione, which is a widely used fungicide.

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Experimental

Reagents and materials

Iprodione, 3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxo-imidazolidine-1-carboxamide (residual pesticide; analysis grade), acetone (pesticide residue and polychlorinated biphenyl; analysis grade), and acetonitorile (HPLC grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Titanium dioxide (~80% anatase form, particle size of 0.1 – 0.3 μm) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

Instruments

Two 10-W black light lamps (FL10BLB-A, peak wavelength of 352 nm) were purchased from Toshiba Lighting and Technology Corporation (Tokyo, Japan). A dry Thermo Unit (DTU-1B) and a Water Bath Shaker (Personal-11) were obtained from Taitec Corporation (Saitama, Japan). The UV irradiation device consisted of the two black-light sources and two mirrors (Fig. 1). PTFE membrane filters (pore size, 0.45 μm) were obtained from SUN-SRi (USA).

A Hewlett-Packard 1050 Series HPLC system equipped with a reversed-phase column (Shiseido CAPCELL PAK C18 UG120, 4.6 mm i.d. × 250 mm, 5 μm) was used to detect iprodione at 230 nm using a UV-Vis detector. The mobile phase was 60% (v/v) acetonitrile containing 4.4 mM trifluoroacetic acid at a flow rate of 0.8 mL min^{-1}. The column temperature was 25˚C. Ten microliters of the test solution were injected using an auto-injector.

Clean-up using titanium dioxide

Commercially purchased dry basil was crushed and passed through a standard sieve (aperture, 425 μm). Five grams of the basil powder were weighed and placed in a glass centrifuge tube, to which 50 mL of acetone was added. The tube was shaken vigorously for 10 min (amplitude, 30 mm; speed, 150 min^{-1}) and centrifuged at 3000 rpm for 5 min to obtain a crude basil extract (supernatant). The crude extract was filtered, and

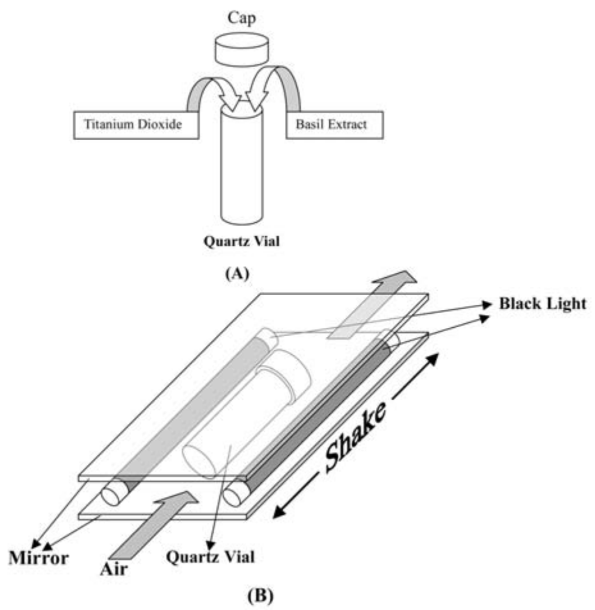

Fig. 1 Illustration of the UV irradiation device. (A) Quartz vial containing basil extract and titanium dioxide. (B) The vial was set parallel between two black-light sources that were placed between the two mirrors.

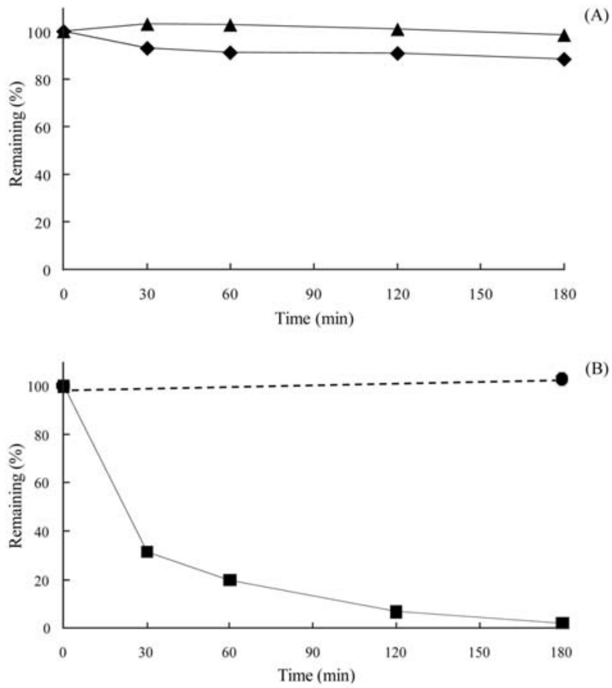

Fig. 2 Effect of UV irradiation on iprodione in the presence of titanium dioxide. (A) Iprodione standard solution with (◆) and without (▲) UV irradiation. (B) Iprodione (●) and interfering peak at 12.7 min (■) in basil extracts. Refer to the text for the reaction conditions.

the extract was obtained. Six grams of titanium dioxide were added to 20 mL of the extract in a 40-mL quartz vial, and the vial was sealed with a septum cap.

The quartz vial containing the mixture was placed between the black light lamps (Fig. 1). The device was shaken (amplitude, 30 mm; speed, 50 min^{-1}), and the quartz vial was irradiated with black light for 180 min. To prevent the temperature of the quartz vial from rising because of heat from the light sources, the device was ventilated continuously. After the extract was irradiated, it was filtered through the membrane filter. An aliquot of the filtrate (1 mL) was placed in a glass test tube, and acetone was evaporated to dryness by nitrogen flow at 38˚C. The residue was dissolved in 1 mL of acetonitrile and centrifuged at 3000 rpm for 5 min. The thus-prepared supernatant was used as the test solution for HPLC analysis.

Results and Discussion

The optimum conditions for the proposed method (*e.g.*, the amount of titanium dioxide, the level of irradiation energy, and time) were assessed. Although the two 10-W black light sources generated a flux of 530 $\mu W/cm^2$, they were insufficient to degrade interfering substances in the extract within 180 min. When the black-light sources were placed between the two mirrors, as shown in Fig. 1, the strength of the UV radiation flux increased to 1085 $\mu W/cm^2$, which was sufficiently strong to reduce the interfering peaks. From these results, the irradiation apparatus shown in Fig. 1 was used in following experiments.

Six grams of titanium dioxide were added to 20 mL of the standard iprodione solution (0.1 – 1.0 ppm in acetone) in a quartz vial, which was then irradiated. The recovery (%) of iprodione in the standard solution was 88.3 ± 1.2% after 180 min of irradiation. On the other hand, when a mixture of iprodione standard and titanium dioxide was shaken for 180 min in the dark, the recovery (%) was 98.5 ± 1.2% (Fig. 2A). This result suggested that iprodione was barely adsorbed on titanium dioxide, although about 12% of the iprodione was decomposed by irradiation for 180 min in the presence of titanium dioxide.

Two hundred microliters of acetone containing 10 – 100 ppm iprodione were added to 20 mL of the dry basil extract. After the addition of titanium dioxide, the mixture was irradiated with black light. The decomposition of interfering substances was monitored by measuring the height of the background at 12.7 min. With a longer irradiation time, the background signal from the interfering substances was significantly reduced (Fig. 3A), while a peak due to iprodione was clearly observed on the chromatogram (Fig. 3B). The recovery of iprodione (1.0 ppm) was 102.6 ± 1.4% (n = 5) after irradiation for 180 min. This result shows that the interfering substances around the peak of iprodione on the chromatogram were almost entirely decomposed. On the other hand, iprodione was quantitatively recovered in the basil extracts (Fig. 3B). However, the iprodione peak height decreased under a prolonged irradiation time greater than 180 min. By this method, the decomposition of the interfering substances was insufficient using less than 6 g of titanium dioxide. On the other hand, excessive amounts of titanium dioxide occasionally formed emulsions during irradiation, and the separation of the extract from titanium dioxide was difficult. Six grams of titanium dioxide are the optimum quantity for 20 mL of the extract.

As a possible reason for the above-mentioned differing results shown in Fig. 3, the different decomposition rates of iprodione and the interfering substances, caused by ultraviolet radiation and titanium dioxide, may be considered. Imidazole, a type of aromatic heterocycle, is reported to have a lower decomposition rate using titanium dioxide.[13] Iprodione contains an imidazolidine structure, which is a type of an imidazole derivative. This may be another reason for the difference. Decomposition with titanium dioxide is due to the difference in

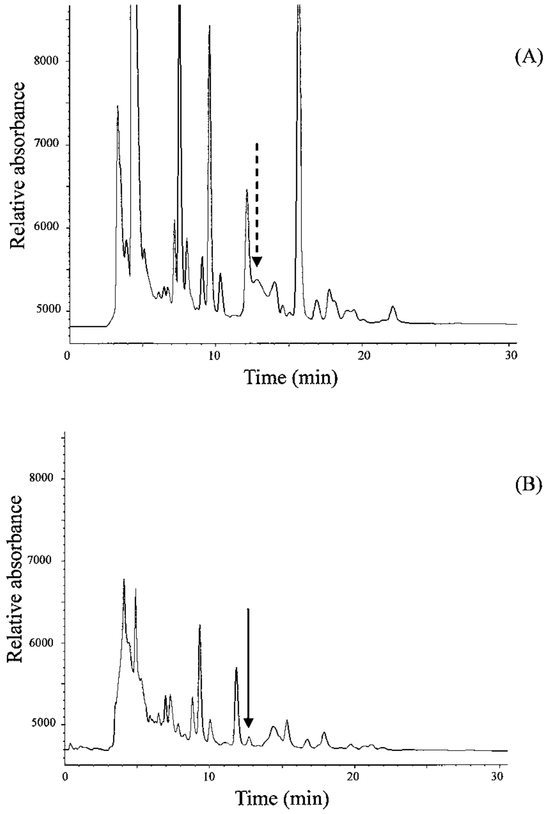

Fig. 3 Typical HPLC chromatograms of the basil extract with titanium oxide (A) before and (B) after UV irradiation for 180 min. The arrow (⇣) shows the retention time of iprodione. The arrow (↓) shows the iprodione peak. Refer to the text for the reaction conditions.

the chemical structure; an examination to obtain the optimum amounts of titanium dioxide and the irradiation time is necessary to apply this method for other types of samples.

We also examined HPLC separation. Gradient elution improved the separation of iprodione and interfering peaks. However, the HPLC analysis time became longer and the peak height of iprodione became smaller than that obtained by isocratic elution.

This study shows the selective decomposition of interfering substances using UV-irradiated titanium dioxide for the HPLC determination of iprodione, a widely used fungicide, in a dry basil extract. Our purification method, which uses titanium dioxide, is simpler than the previous analyses methods for agricultural chemicals.[14]

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