Determination of Hexaconazole in Surface Water Samples from River and the Sea by Liquid Chromatography-Electrospray Tandem Mass Spectrometry

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A simple and selective method was developed for determining the concentration of hexaconazole in river and sea water samples by using liquid chromatography/tandem mass spectrometry with an electrospray ionization interface in the positive ion mode and selective reaction monitoring mode. Trace amounts of hexaconazole were collected in a Sep-Pak Plus tC18 cartridge that was eluted with methanol. The detection limit for hexaconazole was 6 ng/l. The recovery of a standard aqueous solution containing 1 µg/l was 96%. The recovery of hexaconazole in the river and sea water samples was 95% and 90%, respectively. Hexaconazole was not detected in the sea water samples. Trace peaks of hexaconazole were found in the river water samples, the concentration being less than 6 ng/l in all cases. The biological degradation of hexaconazole was tested by using river water. No degradation of hexaconazole was apparent in river water incubated at 20 °C for 3 weeks.

Key words: hexaconazole; liquid chromatography/electrospray tandem mass spectrometry (LC-MS/MS); biodegradation; river water; sea water

The use of agrochemicals for the pre- and post-harvest control of fungal diseases in fruits and vegetables plays an important role in food protection and quality preservation. Several triazolic and pyrimidine derivatives are systemic fungicides used to control a wide range of fungal pathogens on field crops, fruits, and vegetables.

Hexaconazole (Fig. 1; CAS No. 79983-71-4, mw 314.21), a fungicide used on field crops, fruits, and vegetables, has been designated as a Class II Chemical Substance (ADI: 0.0047 mg/kg/day) by the Pollutant Release and Transfer Register (PRTR). The PRTR system has been adopted in various countries, being institutionalized in 1999 in Japan. The PRTR system requires businesses to submit notification of chemical substances they release into the environment. Judging from their physical and chemical properties, Class II Chemical Substances are anticipated to persist in the environment for an extended period of time over a considerable area if the amount manufactured, imported, or used is increased, and thus will pose a risk to the environment. The shipment of hexaconazole has gradually increased from 0.8 t (1991) to 3.4 t (2003) in Japan, and the Japanese Ministry of the Environment has become concerned about its persistence in the environment. The shipment of hexaconazole in Fukuoka Prefecture was the fifth greatest of 47 prefectures in 2004, and hexaconazole has been increasingly used in Fukuoka Prefecture in comparison with other prefectures.

Several methods of liquid chromatography with mass spectrometry (LC-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) for analyzing fungicides or pesticides have been reported. A, B) However, there are few reports of LC-MS/MS methods for

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Abbreviation: LC-MS/MS, liquid chromatography/electrospray tandem mass spectrometry

Fig. 1. Structure of Hexaconazole.
analyzing hexaconazole in environmental water samples.

We describe in this paper, a simple and selective method for the quantification of hexaconazole in surface water samples from rivers and the sea using LC-MS/MS with an electrospray ionization (ESI) interface in the positive ion mode and selective reaction monitoring (SRM) mode. The biological degradation of hexaconazole was also tested in river water.

**Materials and Methods**

**Chemicals and reagents.** Hexaconazole standards were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol and ultrapure water of LC/MS grade were from Wako. HPLC-grade formic acid (98%) was from Kanto Chemical Co. (Tokyo, Japan).

**Sample collection and extraction.** Surface water samples from the river and the sea of northern Kyushu and from the Japan Sea were collected. Water sampling was carried out in June 2006 and October 2006. Each 200 ml water sample was passed through a solid-phase extraction cartridge (preconditioned tC18 with 10 ml of methanol and 10 ml of ultrapure water; Waters, Milford, MA, USA) at a flow rate of 10 ml/min. Hexaconazole was eluted with 6 ml of methanol from the cartridge. After adding 2 ml of ultrapure water to this
methanol solution for adjusting the mobile phase, this solution was analyzed by LC-MS/MS-SRM.

**LC-MS/MS conditions.** Hexaconazole was quantified by LC-MS/MS. Liquid chromatography was performed with an Alliance 2695 instrument (Waters, Milford, MA, USA). A Sun Fire C18 column [150 mm × 2.1 mm i.d., 5 μm (Waters)] was used under an isocratic solvent condition. The mobile phase was 75:25 methanol/0.1% (v/v) formic acid in water, and the flow rate was 0.2 ml/min. The injection volume was 10 μl, and the column temperature was 40 °C.

A Quattro micro API quadrupole mass spectrometer (Waters) with an ESI interface was employed in this study. The interface was operated in the positive ion mode. The capillary and cone voltages were 3.5 kV and 35 V, respectively, source and desolvation temperatures were 80 °C and 350 °C, respectively, and the cone and desolvation gas flows were 501/hr and 6001/hr, respectively. The collision energy was 18 eV, and the SRM mode was used for quantification.

The analysis time for one sample by LC-MS/MS was 15 min.

**Biodegradation test.** Biodegradation was examined by a screening test for the degradation of chemicals in water.19 Water samples from two rivers (St. 1: BOD, 1.1–1.3 mg/l; St. 2: BOD, 2.9–4.2 mg/l, mean values for the years 2003–2005) were used for the microorganism sources; hexaconazole was not detected in these river water samples. In the test, each river water sample and sterile water containing 0.1% (W/V) peptone (50 mg/l initial concentration) was incubated at 20 °C for 3 weeks while shaking in a dark room. River water and sterile water containing 0.1% (W/V) peptone without hexaconazole were similarly incubated as a blank test. The turbidity was measured by observing the optical density at 610 nm to evaluate the antibacterial effects of hexaconazole. The concentrations of hexaconazole were determined at the start, and after 1 d, 3 d, 8 d, and 21 d. After hexaconazole had been extracted with n-hexane from each biodegradation test solution, the concentration was determined by LC-MS/MS.

**Results and Discussion**

**LC-MS/MS results**

Figures 2 and 3 illustrate the precursor ion mass spectrum and product ion mass spectra acquired for 1 mg/l of hexaconazole. [M + H]+ ions were observed in the positive ion scanning mode. The ions were intense at m/z 314 and 316. Hexaconazole has an isotope, its molecular weight presence ratio being [mw 313]:[mw 315] = 100:65.5. The ions at m/z 314 and 316 were selected as precursor ions for collision with argon gas, and their product ions were detected in the product ion scanning mode. The product ion from the precursor ions at m/z 314 and 316 was m/z 70; therefore, m/z 314 → 70 and m/z 316 → 70 were monitored for SRM.
Table 1. Recovery of Hexaconazole in Ultrapure Water, River Water and Sea Water (n = 5)

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Spiking level (ng/ml)</th>
<th>Detected concentration (ng/ml)</th>
<th>Recovery (%)</th>
<th>Precision (R.S.D.)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrapure water</td>
<td>0</td>
<td>n.d.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.96</td>
<td>96</td>
<td>1.8</td>
</tr>
<tr>
<td>River water</td>
<td>0</td>
<td>n.d.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.95</td>
<td>95</td>
<td>0.6</td>
</tr>
<tr>
<td>Sea water</td>
<td>0</td>
<td>n.d.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.90</td>
<td>90</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Fig. 6. Recovery of Hexaconazole from Five Different SPE Cartridges in the Elution of Methanol.

Fig. 7. LC-MS/MS-SRM Chromatograms of the River and Sea Water Samples.
Figure 4 shows the SRM chromatogram for 50 μg/l of hexaconazole.

Calibration curve
The calibration curve for hexaconazole and the concentration correlation coefficients calculated from the peak areas are shown in Fig. 5. The curve shows good linearity in the range 0.5–50 μg/l.

Cartridge for solid-phase extraction
To select the best solid-phase cartridge for the effective and selective investigation of hexaconazole, standard solutions were extracted by using five cartridges: (i) Sep-Pak plus tC18, -SiC18H37; surface functionality (ii) Sep-Pak plus C18, -Si(CH3)2C18H37; surface functionality (iii) Sep-Pak plus PS-2, styrene and divinylbenzene copolymer; surface functionality (iv) Sep-Pak plus AC-2, active carbon; surface functionality and (v) Oasis HLB, N-vinylpyrrolidone and divinylbenzene copolymer (Waters, Milford, MA, USA). Four ml of 0.2 mg/l of a hexaconazole solution was passed through solid-phase cartridges preconditioned with 10 ml of methanol and 10 ml of ultrapure water, and hexaconazole was eluted with methanol from the cartridge. Recovery was detected in aliquots eluted from 0–3 ml, 3–6 ml, and 6–9 ml for each cartridge (Fig. 6). The elution from 0–3 ml showed good recovery (typically >95%) when using three cartridges (tC18, C18 and HLB). The tC18 cartridge was selected for the extraction of hexaconazole because recovery of the eluate from 0–3 ml was 96%, and no hexaconazole was detected in the portion eluted from 3–9 ml.

Detection limit
The method's detection limit (MDL) was determined by using ultrapure water spiked with hexaconazole. Samples of water (2-liter) were spiked with 200 ng of hexaconazole to give a concentration of 0.1 μg/l. MDL was calculated as twice the t-ratio (1.9432; 6 degrees of freedom, 5% level of significance) multiplied by the standard deviation for seven replicate determinations. MDL for this method was 6 ng/l. MDL that the Japanese Ministry of the Environment has demanded for environmental safety is 250 ng/l. Our method therefore satisfies the requirement for sensitive and selective detection of hexaconazole in the environment, while sample collection and extraction with our method are very simple.

Recovery of hexaconazole in ultrapure water, river water, and sea water
The recovery of hexaconazole, expressed as the overall mean from a series of five analyses, is shown in Table 1. The recovery of a standard aqueous solution containing 1 μg/l was 96%, the relative standard deviation being 1.8%. The recovery of hexaconazole in surface water from the river and sea was 95% and 90%, respectively; the relative standard deviation was 0.6% and 7.3%, respectively.

Determination of hexaconazole in the river and sea water samples
Surface water from three different sites of the same river and from three different sites of the bay in Fukuoka Prefecture were sampled in October 2006. Representative SRM chromatograms of the river and sea water samples are shown in Fig. 7. Hexaconazole were not detected in the sea water samples, but trace amounts of hexaconazole were found in all the river water samples, although the concentration was less than 6 ng/l (MDL) in all cases.
Biodegradation of hexaconazole in the river water samples

There have been few reports on the biodegradation of hexaconazole in river water, so we tested this biological degradation in the river water samples. Figure 8a shows the growth of microorganisms in river water containing 0.1% peptone (A, with hexaconazole; B, without hexaconazole) and in sterile water with hexaconazole (C). No antibacterial effect of hexaconazole was apparent in the test. Figure 8b shows the residual concentration of hexaconazole in river water (A-St. 1 and A-St. 2) and sterile water (C) incubated for three weeks. No degradation of hexaconazole in the river water was apparent in this test. The difference in hexaconazole concentration between the river water and sterile water samples after 3–21 d of incubation could have been due to the declining recovery of hexaconazole caused by growing microorganisms. After 3 d, 8 d and 21 d of incubation of the sterile water, St. 1 river water, and St. 2 river water samples containing 0.1% peptone without hexaconazole, hexaconazole was added and then extracted immediately with n-hexane from these solutions, their concentrations being determined by LC-MS/MS (Fig. 8c). The results suggest that the recovery from hexaconazole of river water after 3–21 d of incubation was lower than that from sterile water, and that the difference of hexaconazole concentration between the river water and sterile water samples after incubation could not have been due to biodegradation.

These results of this test indicate that hexaconazole is difficult to degrade and could remain in rivers for a long period. No hexaconazole was detected in the sea water samples, while trace amounts of hexaconazole (less than 6 ng/l (MDL)) were found in the river water samples in Fukuoka Prefecture. However, hexaconazole (more than 6 ng/l) could be detected in surface water samples from the other rivers in Fukuoka Prefecture.

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References