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Application of solid-phase extraction to quantitatively determine cyproconazole and tebuconazole in treated wood using liquid chromatography with UV detection

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Abstract

Solid-phase extraction (SPE) procedures were developed to avoid interference during the quantitative determination of cyproconazole and tebuconazole co-existing in wood extractives. Five species of wood were used, Japanese cedar (*Cryptomeria japonica*), Japanese larch (*Larix leptolepis*), Yezo spruce (*Picea jezoensis*), Sakhalin fir (*Abies sachalinensis*), and western hemlock (*Tsuga heterophylla*). Methanol extractives from the heartwood of all wood samples, except western hemlock, interfered with the quantitative determination of cyproconazole and tebuconazole using liquid chromatography (LC) with UV detection (LC–UV). SPE with Oasis MCX was effective in avoiding this interference. This method also reduced the time and volume of mobile phase required for LC–UV, since wood extractives with long retention times were also removed.

Keywords: Solid-phase extraction; Wood

1. Introduction

Although wood and wood products are attacked by many organisms, principally fungi and termites, they are extensively used in residential construction, decking, and so on. Consequently, when wood is frequently wetted or placed in ground contact it should be treated with preservatives to protect it against wood destroying organisms. Various active ingredients are included in wood preservatives [1], for example, cyproconazole and tebuconazole, which are also widely used as biocides for protecting fruit and vegetable crops. The amounts of such active ingredients in treated wood are specified to guarantee protection. In Japan, the Japan Housing and Wood Technology Center (HOWTEC) specify the amounts of active wood preservative ingredients, including cyproconazole or tebuconazole, in treated wood [2]. Consequently, accurate quantitative determinations of cyproconazole and tebuconazole in wood are needed.

When using the HOWTEC's methods [3] to quantitatively determine the amounts of cyproconazole and tebuconazole in treated wood, the biocides are first extracted with methanol then either liquid chromatography (LC) with UV detection (LC–UV) or gas chromatography with nitrogen-phosphorus detection is carried out. The standard methods of the American Wood-Preservers' Association (AWPA) [4,5] also involve tebuconazole extraction with methanol followed by chromatographic procedures. Since wood contains a large number of compounds that can be extracted with organic solvents, the samples extracted using the above methods include numerous co-extracted components such as wood extractives [6]. Consequently, these extractives might interfere with the quantitative determination of cyproconazole and tebuconazole; particularly as chromatographic analysis is carried out non-selectively. The AWPA's method [4] for tebuconazole determination using LC–UV indicates that changes to some

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of the LC–UV analysis parameters are needed to avoid the interference of wood extractives. However, because the components of these wood extractives vary according to species [6] the LC–UV parameters might also be dependent on species. If these interfering matrix components could be removed from the analytical samples, it would be possible to carry out LC analysis regardless of species.

Solid-phase extraction (SPE) has been widely used as method for preconcentrating and cleaning up analytical samples [7–9]. This method has been previously applied to the determination of cyproconazole and tebuconazole in natural water [10–12] and crops [13]. However, to the best of our knowledge, the quantitative determination of cyproconazole and tebuconazole in treated wood using SPE has not been reported.

Most of the above methods used reversed-phase sorbents such as an ODS or a styrene-divinylbenzene co-polymer for SPE. These sorbents have a relatively low specificity to cyproconazole and tebuconazole. On the contrary, mixed mode sorbents, which have both a reversed-phase and an anion-exchange functional group, can retain lipophilic basic compounds using both mechanisms [7,8]. Zrostlíková et al. [14] used Oasis MCX, a mixed-mode cation exchanger [8,15], to determine tebuconazole and 16 pesticides in fruits. Tebuconazole was successfully recovered from the matrices using Oasis MCX. In this paper, SPE with MCX is applied to determine the amounts of cyproconazole and tebuconazole in treated wood using LC–UV.

2. Materials and methods

2.1. Reagent

Biocide standards with a purity of 95% were provided by Xyence (Tokyo, Japan). High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were purchased from Kanto (Tokyo, Japan). Aqueous ammonium hydroxide solution (28%) was obtained from Kishida Chemical (Osaka, Japan).

2.2. Sample preparation

Spiking solutions were prepared at a concentration of 0.05 mg/ml by weighing 5 mg of cyproconazole or tebuconazole in a 100 ml volumetric flask and diluting with methanol. To construct calibration curves, the spiking solutions were evaporated and diluted with suitable amounts of mobile phase to obtain standard solutions containing cyproconazole or tebuconazole at concentrations of 20, 10, 5, 1 and 0.5 μg/ml, respectively. The standard solutions were filtered through a 0.45 μm membrane filter, and the resulting filtrates were analyzed by LC–UV. The correlations between each biocide peak area and their concentrations were determined by linear regression as $r^2 = 1.0000$ for both biocides.

Heartwoods of Japanese cedar (*Cryptomeria japonica*), Japanese larch (*Larix leptolepis*), Yezo spruce (*Picea jezoensis*), Sakhalin fir (*Abies sachalinensis*), and western hemlock (*Tsuga heterophylla*) were used for preparing methanol extractives of each wood. One gram of each wood was ground with a Wiley mill and extracted with 20 ml of boiling methanol for 2 h. The solutions were filtrated under a vacuum, and the filtrates were concentrated in a rotary evaporator at 40 °C. The concentrated extractives were resolved in 20 ml methanol, or mixed with 2 ml or 0.2 ml of stock solution and then diluted with methanol to 20 ml. Six replicates of the spiked and non-spiked sample solutions were prepared. SPE was applied to three of these replicates and the remaining three were evaporated to dryness. The residues were dissolved in mobile phase and then filtered through a 0.45 μm membrane. The filtrates were analyzed by LC–UV.

2.3. Solid-phase extraction

SPE was carried out using a 3 ml cartridge packed with 60 mg of 30 μm Oasis MCX (Waters, Tokyo, Japan). The cartridges were set on a 12-position SPE vacuum manifold (Supelco, Tokyo, Japan) and conditioned with 2 ml methanol, followed by 2 ml distilled water. After sample loading, the Oasis MCX cartridges were washed with 3 ml of 5% NH_4OH (as 28% aqueous solution) in methanol–distilled water (20:80, v/v) and 3 ml methanol. Finally, the retained compounds were eluted with 28% NH_4OH aqueous solution–methanol (5:95, v/v). All SPE steps were performed at a flow rate of ca 2 ml/min. The eluate was evaporated to dryness and the residue was dissolved in mobile phase and filtered through a 0.45 μm membrane filter. The filtrate was analyzed by LC–UV.

2.4. LC analysis

All LC analyses were performed on an HPLC system consisting of a LC-10AD pump (Shimadzu, Kyoto, Japan), an DIL-10AXL auto sample injector (Shimadzu), a CTO-10AC column oven (Shimadzu), an SPD-10A UV–vis detector (Shimadzu) and a SIL-10A system controller (Shimadzu). The recording and integrating device was a CR-5A Chromatopack (Shimadzu). The LC column used was an Inertsil ODS-3, 5 μm, 150 × 0.6 mm i.d. column (GL Science, Tokyo, Japan). The column temperature was set at 40 °C. The mobile phase was acetonitrile-10 mM phosphate buffer, pH 2.6 (60:40, v/v) with a flow rate of 1.0 ml/min. All samples were injected with 10 μl of mobile phase via the auto sample injector. UV detection was carried out at 220 nm.

3. Results and discussion

3.1. Interference of wood extractives

To confirm the interference of wood extractives on the quantitative determination of cyproconazole and

Table 1
Quantitative determination of cyproconazole and tebuconazole in wood extractives ($n = 3$)

	Spiking level (0.1 mg/g)				Spiking level (0.01 mg/g)			
	Cyproconazole		Tebuconazole		Cyproconazole		Tebuconazole	
	Ratio (%)[a]	S.D.	Ratio (%)[a]	S.D.	Ratio (%)[a]	S.D.	Ratio (%)[a]	S.D.
Japanese cedar	118	2.0	105	0.6	233	13.4	95	26.0
Japanese larch	103	0.4	108	0.7	96	5.0	194	15.8
Yezo spruce	108	1.1	111	1.0	131	0.6	221	2.1
Sakhalin fir	100	0.9	49	2.3	20	2.6	–	–
Western hemlock	101	1.6	101	1.6	102	4.5	110	3.4

[a] Ratio of quantitative amount to spiking amount.

tebuconazole, heartwood from each woody species was used for methanol extractive preparation because most extractives are located here [6]. LC–UV analysis was carried out according to HOWTEC's methods [3] with minor modifications. The details of the LC–UV conditions were described in Section 2.4.

The quantitatively determined amounts of cyproconazole and tebuconazole, which were spiked in extractives of each wood, obtained using LC–UV, are shown in Table 1. At a spiking level of 0.1 mg/g, the ratios of the quantitative amount to the spiking amount for both cyproconazole and tebuconazole in extractives of each wood were above 100%, except for Sakhalin fir. Comparisons of the chromatograms of spiked and non-spiked Sakhalin fir extractives (chromatograms not shown) revealed that the lower result for tebuconazole in the Sakhalin fir matrix was caused by large matrix peaks. The slightly higher results for cyproconazole in the Japanese cedar and Yezo spruce matrices, and for tebuconazole in the Japanese Larch and Yezo spruce matrices were due to an overlapping of the matrix peaks on the biocides peaks. These effects were apparent at a low spiking level, as mentioned below.

At a spiking level of 0.01 mg/g, lower cyproconazole results were obtained and the tebuconazole peak could not be confirmed in the Sakhalin fir extractives. A large matrix peak or high baseline caused these erroneous results, as shown in Fig. 1. On the other hand, excess results were obtained for cyproconazole in the Japanese cedar and Yezo spruce matrices, and for tebuconazole in the Japanese larch and Yezo spruce matrices, since their peaks overlapped or were insufficiently separated from the matrix peaks (Fig. 1). The results for cyproconazole in the western hemlock matrices were not affected at either spiking levels, but the tebuconazole results were slightly higher at a spiking level of 0.01 mg/g.

3.2. Clean-up with solid-phase extraction

Triazole biocides, including cyproconazole and tebuconazole, commonly include a 1,2,4-triazole ring. The nitrogen in this ring can dissociate and the generated cations can be retained by a cation exchanger, such as MCX. On the other hand, most of the wood extractives are probably either retained by MCX with reversed-phase mechanisms or not retained because they are mostly anionic or neutral. Thus, it was considered that the Oasis MCX could separate the triazoles from most of the wood extractives.

By applying cyproconazole and tebuconazole in methanol without wood extractives to SPE on a MCX cartridge showed a recovery of 99% (S.D. = 1.3%, $n = 3$) and 98% (S.D. = 1.1%, $n = 3$) for cyproconazole and tebuconazole, respectively. This result indicates that successful retention of the biocides was

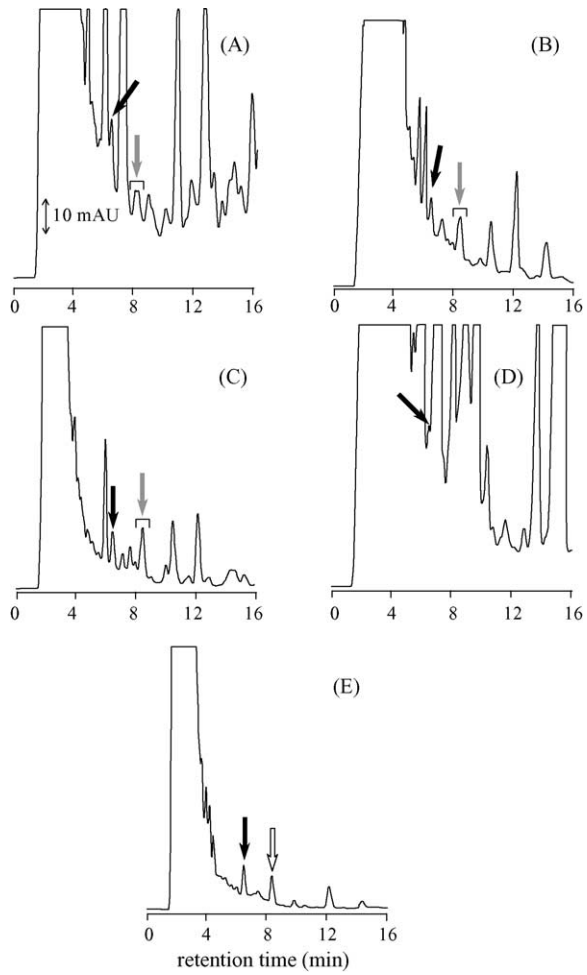

Fig. 1. HPLC chromatograms of cyproconazole and tebuconazole, which were added to the methanol extractives of (A) Japanese cedar, (B) Japanese larch, (C) Yezo spruce, (D) Sakhalin fir, and (E) western hemlock, at a concentration of 0.01 mg/g. Black arrow: cyproconazole, white arrow: tebuconazole, grey arrow: tebuconazole overlapping with the matrix peaks.

Table 2
Average ($n = 3$) recovery of cyproconazole and tebuconazole from wood extractives using SPE with MCX

	Spiking level (0.1 mg/g)				Spiking level (0.01 mg/g)			
	Cyproconazole		Tebuconazole		Cyproconazole		Tebuconazole	
	Recovery (%)	S.D.	Recovery (%)	S.D.	Recovery (%)	S.D.	Recovery (%)	S.D.
Japanese cedar	103	0.4	98	0.6	94	3.5	97	3.4
Japanese larch	100	3.9	98	0.4	94	1.5	99	3.4
Yezo spruce	100	2.1	97	2.0	94	2.1	101	1.9
Sakhalin fir	100	3.5	95	3.4	98	1.1	105	2.6
Western hemlock	99	2.5	94	1.9	102	1.2	103	4.2

achieved when they were loaded as a methanol solution. A previous approach [14] describes the loading of triazoles as an organic solution diluted with water, however, loading without water dilution can avoid precipitation and additional filtration.

Most of the interfering components could be eluted from the sorbents, which were loaded with the extractives, only by washing with methanol. Thus, it can be suggested that these components were retained by the MCX mainly as a result of reversed-phase mechanisms. Subsequently, washing the sorbents with 5% NH_4OH (as 28% aqueous solution) in methanol–distilled water (20:80, v/v) resulted in further cleaning up. Both washing steps were therefore used for SPE. Fig. 2 shows the resultant chromatograms obtained using this method. The cyproconazole and tebuconazole peaks were clearly confirmed while the interfering peaks became significantly decreased.

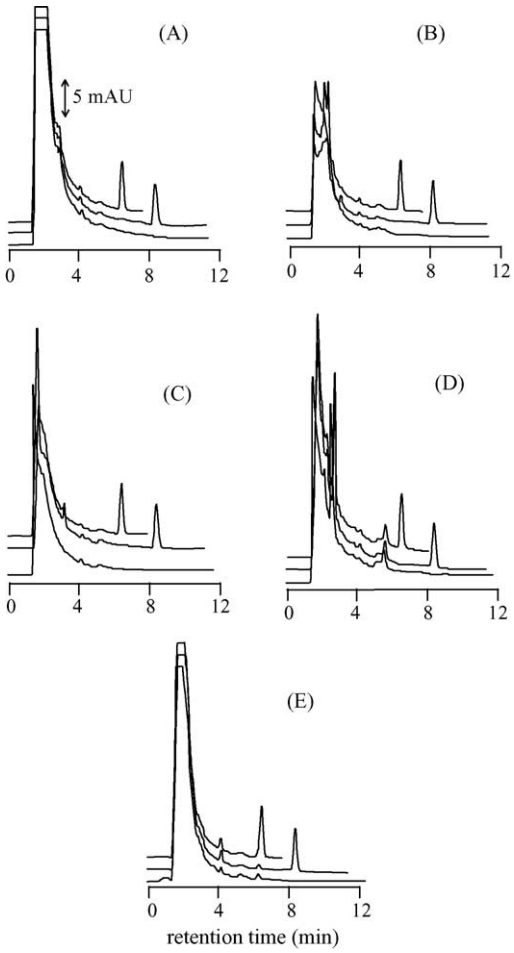

Fig. 2. HPLC chromatograms of the extractives (lower), of the extractives spiked with tebuconazole at a concentration of 0.01 mg/g (middle), and of the extractives spiked with cyprocoanzole at a concentration of 0.01 mg/g (upper), after SPE with MCX. See Fig. 1 for key to panels A–E.

Using this SPE method, the peaks of extractives with long retention times under the present LC conditions were also removed. Thus, this method could reduce the time and volume of mobile phase required for LC analysis. LC analysis was needed for only approximately 8 or 10 min because the retention times of cyproconazole and tebuconazole under such conditions were 6.5 and 8.3 min, respectively.

Table 2 summarizes the recoveries of cyproconazole and tebuconazole from each matrix solution. Satisfactory recoveries were obtained for cyproconazole and tebuconazole at both spiking levels (0.1 and 0.01 mg/g). The effects of the matrices of all wood species on the determination of biocides disappeared. Using the SPE method reported in this paper, quantitative determination of cyproconazole and tebuconazole could be performed by LC–UV analysis without matrix interference.

4. Conclusions

When they co-exist with the biocides, methanol extractives of Japanese cedar, Japanese larch, Yezo spruce and Sakhalin fir heartwoods interfere with the determination of cyproconazole and tebuconazole using LC–UV. SPE with Oasis MCX is effective in avoiding these interferences. With this method, the coexisting biocides in the wood extractives, which contain the interfering components, can be analyzed by LC–UV without changing the LC–UV parameters. Furthermore, this method reduces the time and volume of mobile phase required for LC–UV, since components with long retention times were also removed by SPE with MCX.

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