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ORIGINAL ARTICLE

Quantitative determination of cyproconazole, as a wood preservative, by gas chromatography–mass spectrometry analysis: matrix effect observed in determining cyproconazole and efficacy of adding analyte protectant

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Abstract Quantitative determination of cyproconazole by gas chromatography–mass spectrometry (GC/MS) analysis was investigated. The results suggest that cyproconazole determination is significantly affected by wood extracts. In the presence of methanol extracts of Japanese cedar (*Cryptomeria japonica*) heartwood, cyproconazole showed 50 % larger peak area than that without the extracts. This phenomenon whereby contaminants in the sample solution affect the intensity of the peak signal is known as the matrix effect. An investigation for eliminating the matrix effect revealed that adding sorbitol as an analyte protectant drastically increased the analyte peak intensity and successfully mitigated the matrix effect of the wood extracts. Adding hexaconazole as an internal standard was also useful in reducing the deviation of the data. The efficacy of a novel sample preparation method consisting of adding sorbitol and hexaconazole as analyte protectants and the internal standard, respectively, were applied to the GC/MS analysis of cyproconazole in the presence of wood extracts of the other nine species. It is revealed that cyproconazole can be precisely determined by GC/MS analysis in combination with the analyte protectant and the internal standard.

Keywords Cyproconazole · Wood extracts · GC/MS analysis · Matrix effect · Analyte protectant

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Introduction

Since harvested wood products have been defined as a carbon sink [1, 2], it becomes crucial to prolong the service life of wooden products and delay their emissions of carbon dioxide. One possible method of retarding gas emissions is through wood protection [3], and many investigative studies have been conducted on developing preservatives' performance [4]. Cyproconazole, which is an azole compound fungicide, was found to be an active ingredient in wood preservative in these investigative studies. The compound is now adopted in two wood preservatives defined by the Japanese Industrial Standard K1571:2010 [5] as copper azole (CUAZ) and azole neonicotinoid (AZN) and other standards.

Quantitative determination of cyproconazole has been also developed because the retention of fungicides strongly affects the service life of treated wood. Retention of cyproconazole is usually determined with high-performance liquid chromatography (HPLC) [6, 7]. However, the detector used in the HPLC analysis of cyproconazole, namely a UV monitor, is less selective and wood extracts often hamper cyproconazole determination. To eliminate the influence of wood extracts, a method of cleanup before HPLC analysis was developed using an SCX solid-extraction cartridge [8]. The cleanup method is now adopted in the newest Japanese agricultural standard for sown timber [9].

Although it may be true that the cleanup method is useful to remove the peak hampering the cyproconazole determination, it is also time consuming and increases running costs; hence another simplified determination technique is required.

Unlike less selective detectors of conventional HPLC systems, gas chromatography with mass spectrometry (GC/

MS) systems use highly selective detectors and are hence widely used to analyze numerous pesticides [10]. Therefore, the GC/MS system was considered to determine cyproconazole concentration precisely without solid phase extraction, even in the presence of wood extracts.

In this paper, we describe cyproconazole determination without solid phase extraction using a GC/MS system. We also discuss the matrix effect observed in cyproconazole determination with wood extracts.

Materials and methods

Materials

Cyproconazole and hexaconazole reference standards (>99.0 %, both) and all solvents, namely methanol, acetone, hexane and ethyl acetate, and analyte protectants, namely gluconolactone, gulonolactone and sorbitol, were purchased from Wako Pure Chemical Industries, Ltd., Japan. Cyproconazole stock solution of 10 μg/ml and hexaconazole stock solution (8 μg/ml) were prepared in methanol. Both stock solutions were diluted with methanol to a prescribed concentration.

Wood flours of the Japanese cedar (*Cryptomeria japonica*) sapwood and heartwood were prepared with an IKA Labortechnik A11 basic analytical mill. Wood flours of other species (Table 1), consisting of a mixture of sapwood and heartwood portions, were kindly donated by the Japan Laminated Wood Products Association.

Preparation of methanol extracts of wood flour

One gram of each wood flour and 20 ml of methanol were placed in a screw-cap bottle and sonicated in an ultrasonic bath for 3 h. Following the extraction, part of the supernatant was filtered with a DISMIC-25HP (ADVANTECH) membrane filter and used for further studies.

Table 1 Name of the investigated wood species besides Japanese cedar

Commercial name	Scientific name
Douglas fir	*Pseudotsuga menziesii*
Japanese cypress	*Chamaecyparis obtusa*
Japanese larch	*Larix kaempferi*
Norway spruce	*Picea abies*
Radiata pine	*Pinus radiata*
Siberian larch	*Larix sibirica*
Spruce	*Picea* sp.
Todo fir	*Abies sachalinensis*
Western hemlock	*Tsuga heterophylla*

Sample preparation for GC/MS analysis

The basic scheme of the sample preparation for GC/MS analysis is shown in Fig. 1. One ml of methanol extracts of wood flour and the same volume of a cyproconazole solution were dried under a nitrogen atmosphere below 45 °C. The dried residue was dissolved in 50:50 (v/v) acetone/hexane to remove hydrophilic wood extracts for the following GC/MS analysis (Fig. 1, scheme 1). When using analyte protectants, 2 ml of the acetone/hexane solution was re-dried under the same conditions, and the residue was re-dissolved in 2 ml of methanol containing an analyte protectant and hexaconazole as the internal standard (Fig. 1, scheme 2).

To prepare the GC/MS sample containing wood extracts of the Japanese larch, liquid–liquid extraction was also used except for acetone/hexane extraction. The dried residue was extracted three times with 4 ml of water and the same volume of ethyl acetate at 250 rpm for 10 min. The ethyl acetate fraction was collected and used for GC/MS analysis (Fig. 1, scheme 3).

All solutions for GC/MS analysis were dried with anhydrous sodium sulfate prior to the analysis.

GC/MS conditions

The GC/MS analyses were conducted with a Shimadzu GCMS-QP-2010 Plus under the following conditions: Rtx-5 Amine (RESTEC Corp., USA) capillary column (30 m, 0.25-mm i.d., 0.25-μm film thickness); injection temperature, 250 °C; column temperature from 50 °C (1 min) to 250 °C (0 min) at 30 °C/min, from 250 °C (0 min) to 274 °C (0 min) at 6 °C/min and from 274 °C (0 min) to 300 °C (2 min) at 30 °C/min; injection mode, splitless (pulsed pressure 250 kPa for 1 min); carrier gas, He

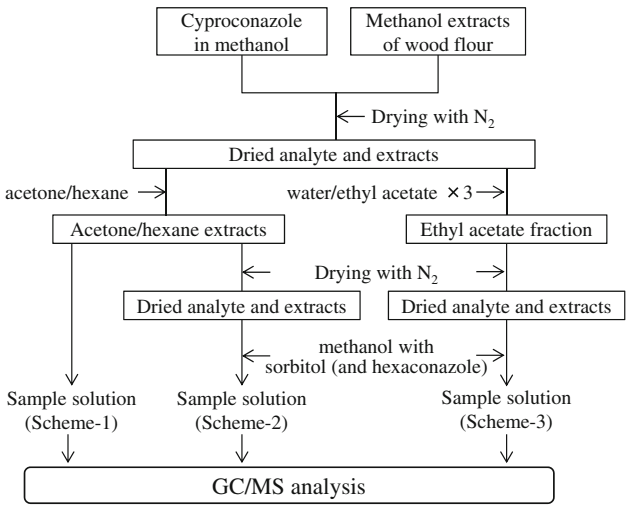

Fig. 1 Scheme for sample preparation

(constant linear velocity at 45.0 cm/s); injection volume, 1 µl; ion source temperature, 230 °C; and interface temperature, 250 °C. Mass range from *m/z* 50 to 350 or *m/z* at 222 and 139 for cyproconazole and those at 214 and 83 for hexaconazole were recorded to monitor signals of these compounds.

Results and discussion

Interference of wood extracts on cyproconazole determination

Figure 2 shows the peak shapes of cyproconazole monitored at *m/z* 222 in the presence and absence of wood extracts of Japanese cedar sapwood and heartwood. The figure suggests that no wood extract appears at the same retention time of cyproconazole. Since no peak looked overlapping that of cyproconazole, the peak area of cyproconazole with the extracts was compared to that without the extracts. The result of the comparison is shown in Fig. 3. In this comparison, the cyproconazole concentration in the original solution was set to 0.625 ppm, a value similar to that obtained from Japanese cedar specimens treated with CUAZ at K2 level [9], and diluted to ninefold with 50:50 (v/v) acetone/hexane because of decreasing wood extract amount, especially with high hydrophilicity.

As shown in the figure, the sample solution diluted to one-ninth of the original showed a 50 % higher peak area with wood extracts of Japanese cedar heartwood compared to that without the extracts. This result suggests that cyproconazole determination could be significantly affected by the methanol extracts of Japanese cedar. This phenomenon, whereby contaminated compounds influence the analyte peak area, is known as a matrix effect and is attributed to contaminated compounds interacting with active sites in the GC/MS system, thus decreasing degradation, adsorption, or both of the coinjected analyte [11–13].

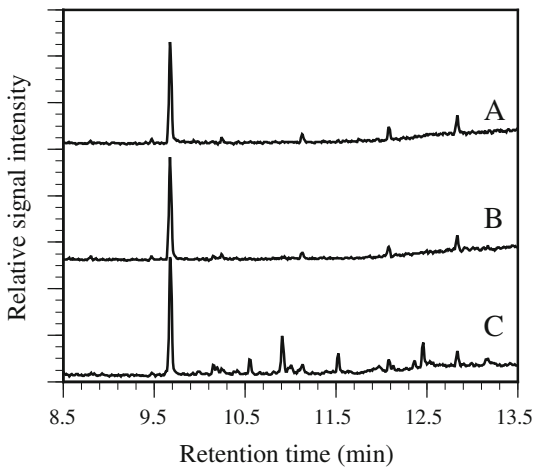

Fig. 2 Signal intensity monitored at *m/z* 222 with and without methanol extracts of Japanese cedar. The sample solution was prepared according to scheme 1 in Fig. 1. One ml of the solution contained 0.0625 µg cyproconazole and wood extracts from 5 mg of Japanese cedar wood flour. *a* Cyproconazole without wood extracts, *b* cyproconazole with sapwood extracts, *c* cyproconazole with heartwood extracts

Reducing matrix effects

To eliminate the matrix effects, the following methods have been proposed: (1) reducing the matrix concentration, (2) using the standard addition method and (3) using analyte protectants [11–13].

We first investigated reducing the matrix concentration by dilution, the result of which is also shown in Fig. 3. This result indicates that dilution decreases the difference among peak areas with and without wood extracts. However, it also suggests that the dilution is insufficient to completely eliminate the matrix effects of wood extracts.

Next, we tried to investigate the potential of the standard addition method. However, we found that the standard addition method could not be adopted to determine cyproconazole by GC/MS analysis, since no linear relation was observed between the cyproconazole concentration and the peak area of the analyte (Fig. 4) [14].

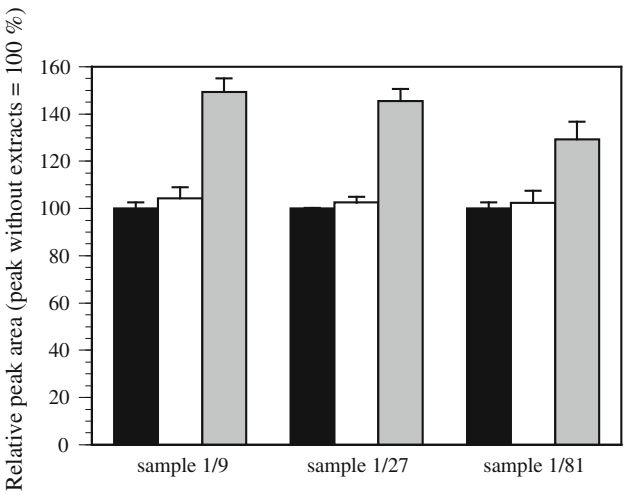

Fig. 3 Cyproconazole peak area with and without methanol extracts of Japanese cedar. 1 ml of original solution, containing 0.625 µg cyproconazole and wood extracts of 50 mg wood flour, was prepared according to scheme 2. The solution was dried and then diluted with 9–81 ml of acetone/hexane ($n = 2$). *Black, white and gray bars* indicate mean peak areas of the samples without wood extracts, with wood extracts of Japanese cedar sapwood and those of heartwood, respectively. The *suffix* of the sample name indicates the dilution factor. Samples with suffixes 1/9, 1/27 and 1/81 were prepared from 1 ml of the dried original solution by dilution to 9, 27 and 81 times the volume of 50:50 (v/v) acetone:hexane, respectively. *Error bars* indicate standard deviations

Finally, we investigated adding analyte protectants, which are defined as compounds that strongly interact with active sites in the GC/MS system, thus decreasing the degradation, adsorption or both of coinjected analytes. Several research groups proposed combinations of analyte protectants that cover the whole range of pesticides with low to high boiling points [11–13]. However, in contrast to their approaches, we investigated the efficacy of adding each analyte protectant because our target compound was cyproconazole alone. With reference to these studies, we chose gluconolactone, gulonolactone and sorbitol as analyte protectants and tested the efficacy of each on the cyproconazole determination. The result showed the peak area of the sample with analyte protectants expanding to twice the size of that without the protectant, showing that all analyte protectants were effective (Fig. 5). Among these protectants, sorbitol looked most effective in improving peak intensity, while the solubility of sorbitol in methanol looked better than the other protectants; hence, sorbitol was selected as the analyte protectant for further studies.

Effect of sorbitol addition on cyproconazole determination

The relation between cyproconazole concentration and peak area in the presence of sorbitol is shown in Fig. 6. A linear relation (correlation coefficient = 0.999) can be observed, despite the absence of the same when no analyte protectant is present (Fig. 4), which clearly indicates that sorbitol successfully protected cyproconazole from degradation and adsorption in the GC/MS system.

The effect of sorbitol addition on removing the matrix effects is shown in Fig. 7a. It is revealed that the matrix effects of Japanese cedar heartwood extracts can be considerably eliminated by adding sorbitol. The ratios of the peak area with the heartwood extracts against that without the extracts were 1.13 for cyproconazole concentration at 62.5 ng/ml to 1.04 for cyproconazole concentration at 1 μg/ml. Figure 7b also shows the effects of sorbitol addition. In the case of Fig. 7b, 160 ng/ml hexaconazole as an internal standard was added with the analyte protectant to correct an error caused by the dispersion of the GC/MS system, namely the dispersion of injection volume and that of the detector's sensitivity. The

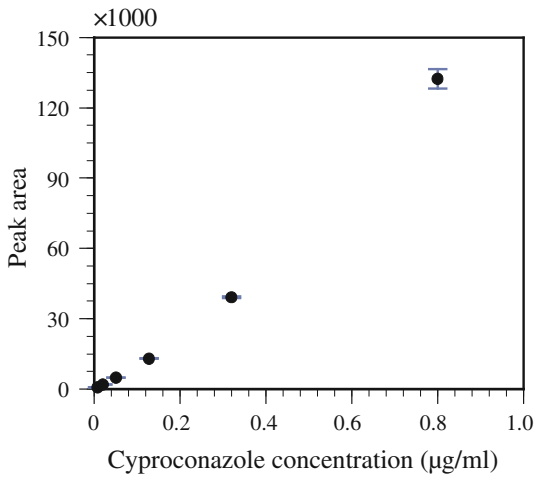

Fig. 4 Relation between cyproconazole concentration and peak area. Sample solutions were prepared according to scheme 1 without wood extracts ($n = 4$). *Bars* indicate standard deviations

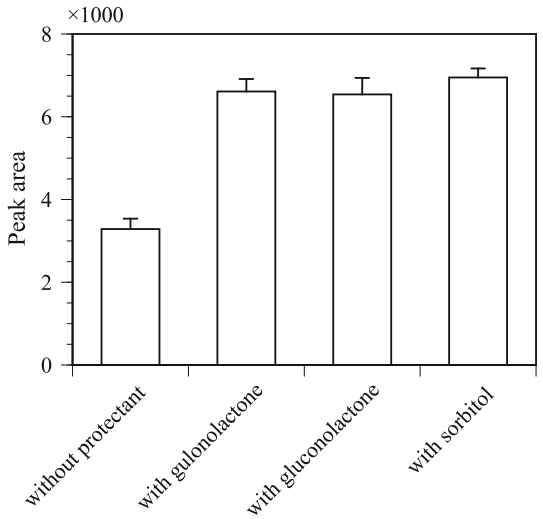

Fig. 5 Influence of analyte protectants on the cyproconazole peak area. Sample solutions containing 0.0625 μg/ml cyproconazole and each of analyte protectants 1 mg/ml were prepared according to scheme 2 ($n = 4$). *Error bars* indicate standard deviations

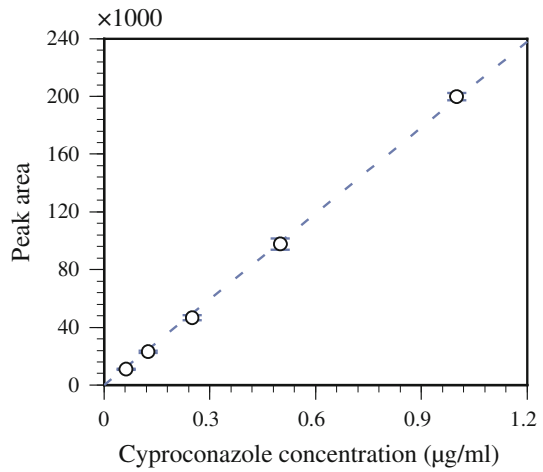

Fig. 6 Relation between cyproconazole concentrations and peak areas in the presence of 1 mg/ml sorbitol. Sample solutions of various concentrations of cyproconazole were prepared according to scheme 2 ($n = 4$). The *broken line* indicates the linear square fit of peak areas against cyproconazole concentrations given by the following equation: peak area = 198 × cyproconazole concentration (ng/ml). *Bars* indicate standard deviations

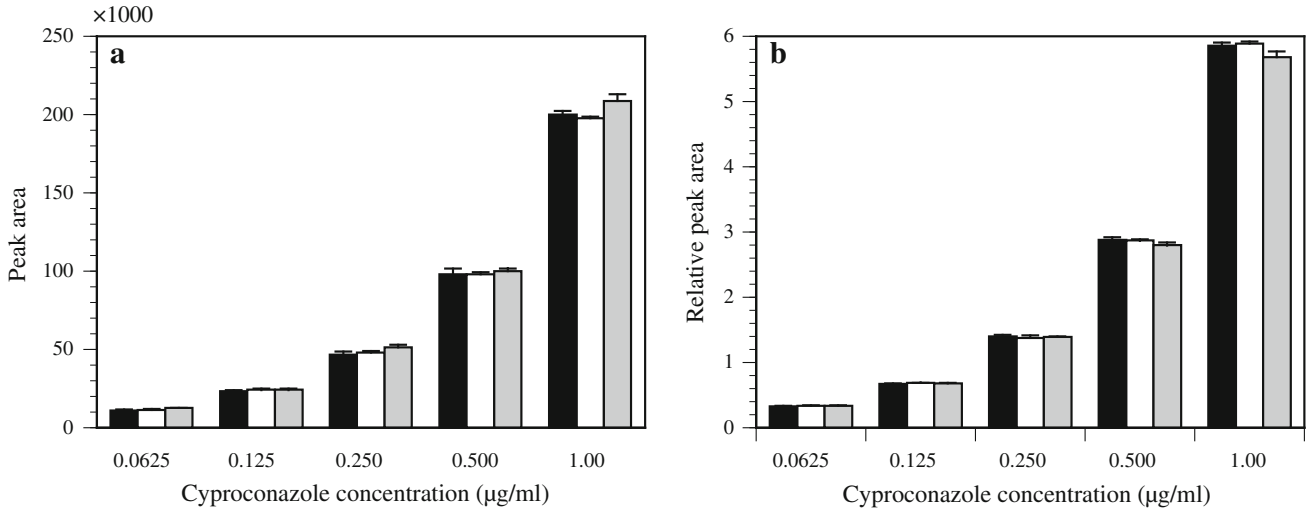

Fig. 7 Effect of sorbitol addition on cyproconazole determination in the presence of methanol extracts of Japanese cedar. Sample solutions containing 0.0625–1.0 μg/ml cyproconazole, 1 mg/ml sorbitol and 0.16 μg/ml hexaconazole were prepared according to scheme 2 ($n = 4$). **a** Peak areas of cyproconazole were directly compared. **b** Ratios of cyproconazole and hexaconazole peak areas were compared. *Black, white and gray bars* indicate the sample without wood extracts, that with sapwood extracts and that with heartwood extracts, respectively. Ratios of wood flours against sample solutions were the same as that in Fig. 2. *Error bars* indicates standard deviations

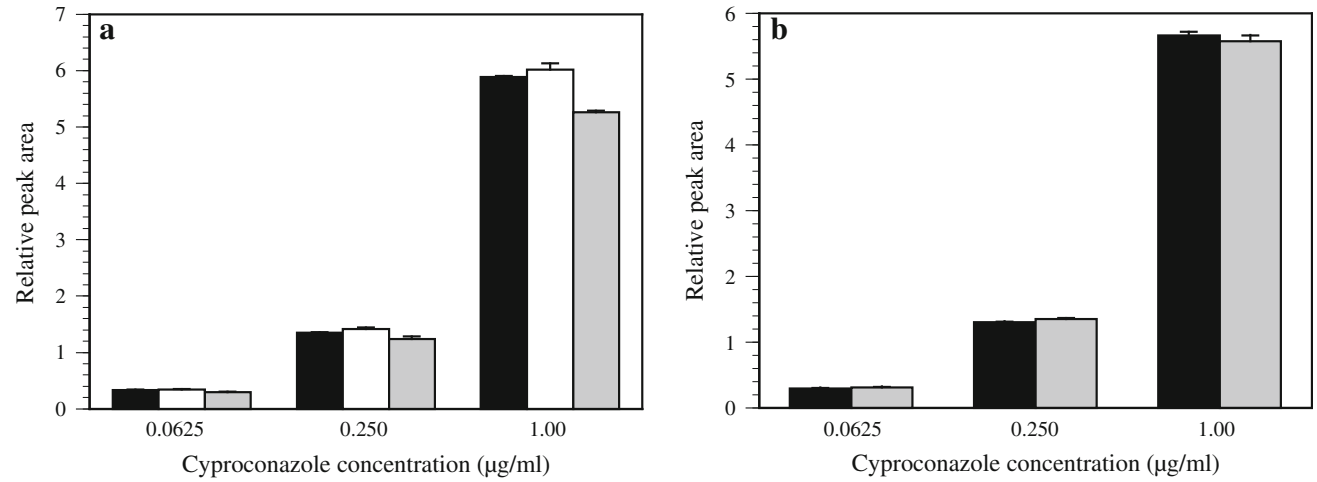

Fig. 8 Effect of sorbitol addition on cyproconazole determination in the presence of methanol extracts of various wood species. Sample solutions containing 0.0625–1.0 μg/ml cyproconazole, 1 mg/ml sorbitol and 0.16 μg/ml hexaconazole were prepared. Ratios of the peak areas of cyproconazole against those of hexaconazole were compared. **a** Sample solutions were prepared according to scheme 2 in Fig. 1. *Black, white and gray bars* indicate the sample without wood extracts ($n = 2$), that with wood extracts of various wood species listed in Table 1 except Japanese larch ($n = 2$ for each species) and that with those of Japanese larch ($n = 2$), respectively. Ratios of wood flours against sample solutions were same as that in Fig. 2. **b** Sample solutions were prepared according to scheme 3 in Fig. 1. *Black and gray bars* indicate the sample without wood extracts and with Japanese larch extracts, respectively ($n = 3$). *Error bars* indicate standard deviations

ratios of the cyproconazole peak area with extracts against that without extracts ranged between 0.97 and 1.03. This indicates that cyproconazole can be precisely determined by GC/MS analysis via a combination of using sorbitol as the analyte protectant and hexaconazole as the internal standard. Since substituting hexaconazole for tebuconazole proved unhelpful for the precise cyproconazole determination (data not shown), using hexaconazole that has similar retention time (Rt 9.3 min) to cyproconazole could be important to facilitate such precise determination.

Effects of sorbitol addition on the cyproconazole determination with methanol extracts of the other 9 wood species are shown in Fig. 8a. Except for the peak area of Japanese larch, all peak areas are almost the same as those without wood extracts. The coefficient of variation of the peaks without Japanese larch was less than 3 %, showing

that GC/MS analysis with sorbitol and hexaconazole is useful in determining cyproconazole impregnated into other wood species.

The reason why cyproconazole with wood extracts of Japanese larch showed a lower peak area appeared that hydrophilic compounds such as arabinogalactan extracted from the Japanese larch aggregated with cyproconazole when cyproconazole and larch extracts were dried and re-dissolved in 50:50 (v/v) acetone/hexane. To confirm the above hypothesis, dried cyproconazole and hydrophobic wood extracts of larch were extracted by liquid–liquid extraction with water and ethyl acetate by preventing aggregation. The analyte and the matrix extracted in the ethyl acetate fraction were initially dried and subjected to GC/MS analysis with a methanol solution containing sorbitol and hexaconazole. Liquid–liquid extraction gave the same peak area as that of the reference sample (Fig. 7b), which suggests that hydrophilic extracts could interfere with the cyproconazole determination as in Fig. 7a.

These results indicate that cyproconazole can be precisely determined by GC/MS analysis in combination with the analyte protectant and the internal standard. Our next goal is optimization of the extraction procedure of cyproconazole from wood flour, because the conventional extraction solvent, methanol, is so hydrophilic that the extracts contain various compounds unsuitable for GC/MS analysis. It is necessary to remove these compounds prior to GC/MS analysis as long as methanol is used as the extraction solvent. If hydrophobic solvents become available for extraction, cyproconazole determination by GC/MS analysis will be simplified furthermore.

Conclusions

Cyproconazole determination using a GC/MS system was investigated and the following conclusions were obtained.

1. A detector of GC/MS system is selective enough to distinguish the peak signal of cyproconazole from those of wood extracts.
2. Matrix effects are observed in cyproconazole determinations with wood extracts. Wood extracts in the sample solutions significantly increase the cyproconazole peak areas.
3. The matrix effects are partially mitigated by dilution of the sample solution.
4. The matrix effects by wood extracts can be absolutely removed by adding sorbitol as the analyte protectant and hexaconazole as the internal standard to the sample solution.
5. Liquid–liquid extraction is useful in case that cyproconazole is lost during sample preparation by precipitation of cyproconazole with hydrophilic wood extracts.

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