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High-performance liquid chromatography with dual-wavelength ultraviolet detection for measurement of hinokitiol in personal care products

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Synopsis

Hinokitiol is found in the heartwood of several cupressaceous plants and is frequently added to cosmetic products such as hair restorers, skin lotions, and body soaps because of its potent and broad-spectrum antibacterial activity. In this study, we established a simple method of hinokitiol determination by high-performance liquid chromatography (HPLC) with dual-wavelength ultraviolet detection at 240 and 345 nm, using a reversed-phase C_4 column (RP-4). The retention time of hinokitiol was 7.1 min at both wavelengths. The value of the symmetry coefficient of the hinokitiol peak was close to 1 when the RP-4 column, not an RP-8 or RP-18 column, was used. With the RP-4 column, the regression equation for hinokitiol showed good linearity in the range of 0.05–5 μg/ml, with a detection limit (signal-to-noise ratio of 3) of 0.005 μg/ml at 240 nm and 0.01 μg/ml at 345 nm. The coefficients of variation at 240 and 345 nm were less than 8.2% and 8.7%, respectively, and the recovery was good. The proposed method was used for the determination of hinokitiol in commercial hair restorers, skin lotions, and body soaps.

INTRODUCTION

Hinokitol (Figure 1, β-thujaplicin, 4-isopropyl-2-hydroxycyclohepta-2,4,6-trien-1-one) is a naturally occurring toxic compound belonging to the class of tropolones that contain an unsaturated seven-membered carbon ring. The compound is found in the heartwood of several cupressaceous plants, such as western red cedar (*Thuja plicata*), eastern white cedar (*Thuja occidentalis*), hinoki cypress (*Chamaecyparis obtusa*), and hiba (*Thujopsis dolabrata*) (1,2). Since hinokitiol has potent antibacterial and antifungal activities (minimum inhibitory concentration of 0.2 μg/ml for *Staphylococcus epidermidis* and *Daedalea dickinsii*) (2,3), it is added to products such as hair lotions, skin lotions, and body soaps, among other cosmetics.

Little work has been reported on the gas chromatography (GC) or high-performance liquid chromatography (HPLC) of hinokitiol because the tropolone ring has a chelating

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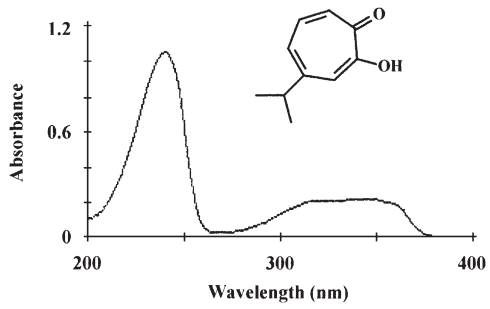

Figure 1. Chemical structure and UV absorption spectrum of hinokitiol.

property, is unstable to heat, and tends to be adsorbed on the stationary phase. Quantitative determinations of hinokitiol by GC and capillary GC have been performed after derivatization with trimethylsilyl chloride and with diazomethane, respectively (1,4). Hanafusa *et al.* (5) reported the determination of hinokitiol in cosmetics by HPLC with ultraviolet (UV) detection following the formation of hinokitiol-copper(II) complex by addition of copper(II) to the mobile phase. However, the sensitivity of these methods was not discussed. Further, it is time-consuming and laborious to treat waste containing copper(II). A sensitive HPLC determination of hinokitiol based on the formation of difluoroborane compounds was reported to have a detection limit of 40 pg of hinokitiol (6). Recently, hinokitiol was determined using a capillary zone electrophoresis method, which provided a detection limit of 0.21 μM (7).

In this paper, we compared the HPLC determination of hinokitiol with RP-4, RP-8, and RP-18 columns. The method using RP-4 was applied for the measurement of hinokitiol levels in a hair restorer, a skin lotion, and a body soap.

EXPERIMENTAL

APPARATUS

The HPLC system comprised a model LC-10AT pump (Shimadzu, Kyoto, Japan), a Rheodyne injection valve (Cotati, CA, USA) with a 200-μl loop, and a model SPD-10A dual UV detector (Shimadzu) operated at 240 and 345 nm. The HPLC columns (150 mm × 4.6 mm, Mightysil® RP-4, RP-8, and RP-18 GP, Kanto Chemical, Tokyo, Japan) were packed with 5-μm particles of C_4, C_8, and C_{18} packing materials, respectively. Quantification of the peaks was performed with a Chromatopac Model C-R8A integrator (Shimadzu).

REAGENTS

Hinokitiol was obtained from Tokyo Chemical Industry (Tokyo, Japan). General reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan). The tested hair restorer, skin lotion, and body soap were purchased from markets.

UV ABSORPTION SPECTRUM

The UV absorption spectrum of hinokitiol was obtained with a UV-1200 spectrophotometer (Shimadzu). Hinokitiol (5 μg/ml) was dissolved in the mobile phase for HPLC analysis using RP-4 as described below, and the spectrum was determined in the range of 200 to 400 nm.

PROCEDURES

RP-HPLC procedure. The mobile phases for HPLC analysis using RP-4, RP-8, and RP-18 consisted of acetonitrile:water:trifluoroacetic acid (300:700:0.5, 450:550:0.5, and 500:500:0.5, respectively, v/v/v). The samples were eluted from the column at a flow rate of 1.0 ml/min at room temperature.

Preparation of standard solutions. A stock solution of hinokitiol (160 μg/ml) in 50% ethanol was prepared and diluted with water as required. It was stored in a dark glass bottle at 4°C. The working concentrations of hinokitiol were set at 0, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 μg/ml. Aliquots of 200 μl were injected into the HPLC column.

Sample preparation. The tested hair restorer (0.50 ml), skin lotion (10 ml), and body soap (0.10 g) were each diluted to 100 ml with water, and aliquots (200 μl) were directly injected into the column.

Recovery. The known amounts of hinokitiol (0.250 mg, 80.0 μg, and 16.0 μg) were added to the hair restorer (0.50 ml), skin lotion (10 ml), and body soap (0.10 g), respectively, and were diluted as the same manner. Aliquots (200 μl) were analyzed and hinokitiol levels were determined in each sample. The recovery value was calculated as follows.

$$\text{Recovery value} = \frac{(\text{Measured amount after addition}) - (\text{Known amount})}{(\text{Measured amount before addition})} \times 100$$

RESULTS AND DISCUSSION

UV ABSORPTION SPECTRUM

The UV absorption spectrum of hinokitiol (Figure 1) showed absorption maxima at around 240 and 345 nm. Therefore, hinokitiol was monitored at 240 and 345 nm to maximize the sensitivity.

CHROMATOGRAMS

Figure 2 shows typical chromatograms of hinokitiol (1 μg/ml) using RP-18, RP-8, and RP-4 columns. The retention times were 5.0, 6.2, and 7.1 min, respectively, at both wavelengths. The symmetry coefficients at 240 nm and 345 nm were 3.1 and 3.7 for RP-18, 1.9 and 1.7 for RP-8, and 1.3 and 1.3 for RP-4, respectively.

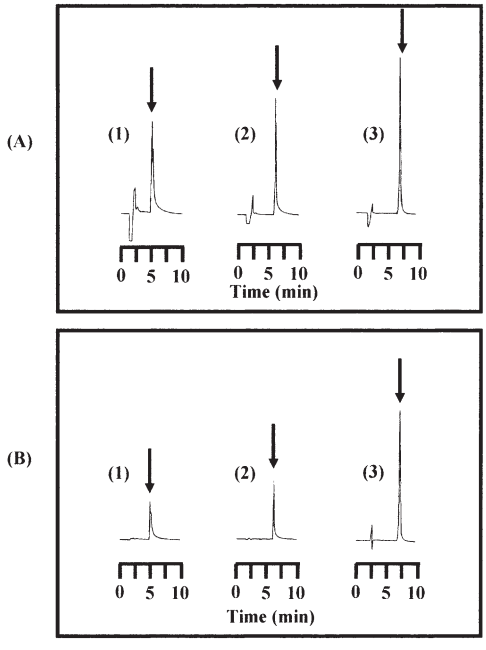

Figure 2. Typical chromatograms of hinokitiol in a standard sample (1 μg/ml), obtained with RP-18, RP-8, and RP-4 columns. (A) 240 nm; (B) 345 nm. (1) RP-18; (2) RP-8; (3) RP-4. Arrowed peak: hinokitiol.

METHOD VALIDATION

Linearity. As shown in Figure 3, standard curves of hinokitiol on the RP-4 column, detected at 240 and 345 nm, were constructed by plotting integrated peak area vs hinokitiol concentration (0.05–5 μg/ml). Linear relationships were obtained: y = 667x + 15.3 at 240 nm and y = 265x + 17.5 at 345 nm. The squared regression coefficients (r^2) at 240 and 345 nm were 0.9974 and 0.9933, respectively.

Sensitivity. The detection limits at 240 and 345 nm were established to be 0.005 (1 ng, 0.03 μM) and 0.01 (2 ng, 0.06 μM) μg/ml, respectively (S/N = 3). Since previous methods provided detection limits of 40 pg and 0.21 μM of hinokitiol (6,7), our method is intermediate in terms of sensitivity.

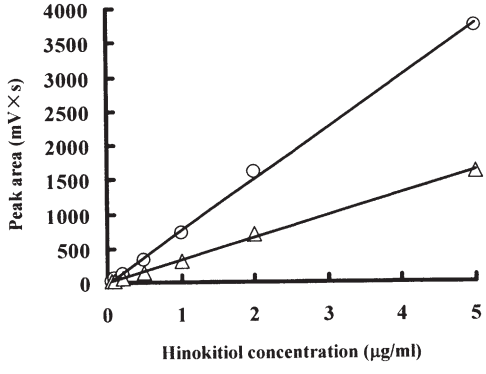

Figure 3. Standard curves of hinokitiol. (○) at 240 nm. (△) at 345 nm.

Table I
Intraday Assay Reproducibility for Determination of Hinokitiol

Concentration (μg/ml)	Measured (μg/ml) (mean ± S.D., n = 5)	C.V. (%)	Recovery (%)
240 nm			
0.05	0.0497 ± 0.0029	5.8	99.4
0.5	0.509 ± 0.027	5.3	101.8
5	5.12 ± 0.21	4.1	102.4
345 nm			
0.05	0.0515 ± 0.0035	6.8	103.0
0.5	0.508 ± 0.024	4.7	101.6
5	5.06 ± 0.19	3.8	101.2

PRECISION AND ACCURACY

Precision and accuracy for intra- and interday assays are shown in Tables I and II. In the intraday assay (Table I), the ranges of standard deviation (S.D.) from the average (C.V.) of hinokitiol at 240 and 345 nm were within the ranges of 4.1–5.8% and 3.8–6.8%, respectively. The recoveries were in the range of 99.4–103.0%. In the interday assay (Table II), the corresponding values were within the ranges of 4.4–8.2% and 4.7–8.7%, respectively, and the recoveries were in the range of 96.6–104.4%.

APPLICATION TO THREE PERSONAL CARE PRODUCTS

The hinokitiol levels in the skin lotion and body soap used in this study were not specified, while the label concentration for the hair restorer was 0.5 mg/ml. Each product was examined six times with our newly developed method. Typical chromatograms are shown in Figure 4. For the hair restorer, the average values of hinokitiol level with detection at 240 nm and 345 nm were 0.536 mg/ml (C.V. = 4.1%, recovery = 107.2%) and 0.502 mg/ml (C.V. = 3.9%, recovery = 100.4%), respectively. Recovery was satisfactory. The corresponding results were 7.81 μg/ml (C.V. = 6.7%) and 8.02 μg/ml (C.V. = 6.8%) for the skin lotion and 0.182 mg/g (C.V. = 7.2%) and 0.178 mg/g (C.V. = 7.4%) for the body soap, respectively.

In addition, recovery values of diluted samples spiked with a known amount of hinokitiol were examined at 240 and 345 nm. The known amount of hinokitiol (0.250 mg, 80.0 μg,

Table II
Interday Assay Reproducibility for Determination of Hinokitiol

Concentration (μg/ml)	Measured (μg/ml) (mean ± S.D., n = 5)	C.V. (%)	Recovery (%)
240 nm			
0.05	0.0494 ± 0.0033	6.7	98.8
0.5	0.514 ± 0.042	8.2	102.8
5	5.22 ± 0.23	4.4	104.4
345 nm			
0.05	0.0520 ± 0.0045	8.7	104.0
0.5	0.483 ± 0.037	7.7	96.6
5	5.14 ± 0.24	4.7	102.8

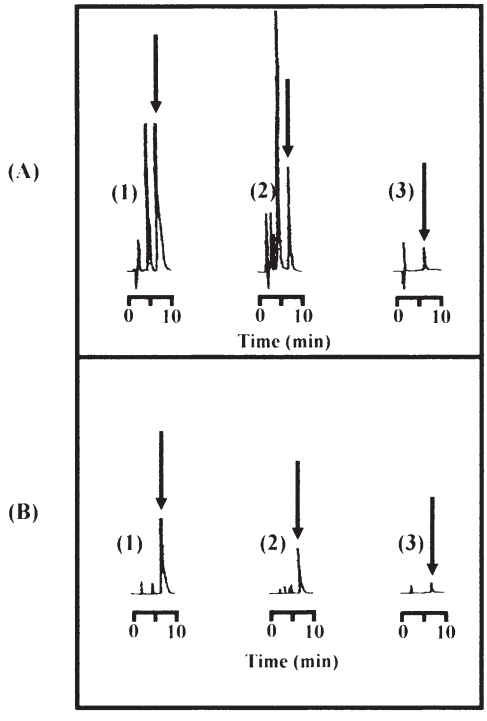

Figure 4. Typical chromatograms (on RP-4) of hinokitiol in hair restorer, skin lotion, and body soap. (A) 240 nm; (B) 345 nm. (1) Hair restorer; (2) skin lotion; (3) body soap. The tested samples were diluted with water as described in "Procedures." Arrowed peak: hinokitiol.

and 16.0 μg) was added to the hair restorer (0.50 ml; 0.268 mg at 240 nm and 0.251 mg at 345 nm), the skin lotion (10 ml; 78.1 μg at 240 nm and 80.2 μg at 345 nm), and the body soap (0.10 g; 18.2 μg at 240 nm and 17.8 μg at 345 nm), respectively. The mixtures were diluted and analyzed. As shown in Table III, the recovery values were satisfactory (92.4–104.8%). These results indicate that our method is capable of measuring hinokitiol concentration in hair restorers, skin lotions, and body soaps.

Table III
Recovery of Hinokitiol in Hair Restorer, Skin Lotion, and Body Soap

Samples	Recovery (%, mean ± S.D., n = 5)
Hair restorer	
At 240 nm	102.2 ± 6.8
At 345 nm	104.8 ± 6.6
Skin lotion	
At 240 nm	96.4 ± 6.3
At 345 nm	95.0 ± 6.2
Body Soap	
At 240 nm	92.4 ± 5.3
At 345 nm	94.6 ± 5.5

CONCLUSION

We present a simple HPLC method with dual-wavelength UV detection for determination of hinokitiol, using an RP-4 column. The sensitivity of our method was moderate, but was sufficient for the measurement of hinokitiol levels in the tested personal care products.

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