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High-performance liquid chromatographic method for determination of dehydroabietic and abietic acids, the skin sensitizers in bindi adhesive

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Abstract

A sensitive and selective high-performance liquid chromatographic (HPLC) method was developed for simultaneous determination of two common allergens, dehydroabietic (DHAA) and abietic acid in bindi adhesive with fluorimetric and ultraviolet (UV) absorbance detection. Bindi is a cosmetic item used by Indian women on their foreheads. The sample was ultrasonicated with 1 ml of acetonitrile for 30 min. A 0.6-ml aliquot of the extract was mixed with an equal volume of deionized water. After centrifugation, 1 ml of the supernatant was percolated through a preconditioned C_{18} solid-phase extraction column. After rinsing the column with 1 ml of acetonitrile–water (60:40, v/v), the analytes were eluted with 1 ml of mobile phase A and 50 μl of the eluent was used for HPLC analysis. The two mobile phases used for gradient elution were: (A) methanol–water (90:10, v/v) containing 0.06% (v/v) formic acid and (B) methanol–water (75:25, v/v) with 0.1% (v/v) formic acid. The flow-rate was set at 1.0 ml/min. DHAA was detected with fluorescence (excitation 225 nm and emission 285 nm) at 3.6 min. Abietic acid was detected at 6.1 min with UV at 238 nm. The lowest detection limits (signal-to-noise ratio 3) were 0.5 and 1.25 ng for DHAA and abietic acid, respectively. Analytical recovery and reproducibility generally exceeded 95 and 90%, respectively. DHAA and abietic acid were present in most of the bindi samples tested, with mean values 0.36 μg of DHAA ($n = 26$) and 0.31 μg of abietic acid ($n = 24$) per sample.

1. Introduction

Rosin (colophony) is a complex mixture of many compounds. It contains about 90% resin acids and 10% neutral matter. Owing to its very strong adhesive effect, it is commonly found in glues and adhesives. Resin acids found in rosin are generally of the abietic and pimaric types. Of these, the abietic type acids are easily oxidized and allergenic. Dehydroabietic acid (DHAA) and abietic acid are the major acids of the abietic type found in different types of rosin [1,2].

Bindi is a cosmetic item used by Indian women on their foreheads to enhance their appearance and as a mark of marital status. It usually originates from the Indian subcontinent. A patient with contact dermatitis to bindi adhesive was found to be sensitive to colophony on patch testing. The identification and confirmation of the presence of these allergens in bindi would provide useful information for patient management. Therefore, a method was developed to

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measure the amount of DHAA and abietic acid in samples of bindi.

DHAA and abietic acid have previously been analyzed, both by gas chromatography [3–5] and by high-performance liquid chromatography (HPLC) [1,6–8]. The most recent HPLC method with ultraviolet (UV) absorbance detection enabled the determination of 0.015% of DHAA and 0.001% of abietic acid in 200 mg of contact adhesive [7]. However, this method was found to be insensitive and non-specific. In the present study, a gradient elution method was developed for baseline separation of DHAA and abietic acid in bindi adhesives. We used solid-phase extraction (SPE) for sample cleaning prior to HPLC analysis. This procedure eliminates matrix interferences and enhances the specificity of detection. The optimum detection conditions for DHAA and abietic acid using fluorimetry and UV spectrometry were also investigated. Measurement of DHAA by the fluorimetric method was at least 100 times more sensitive than by UV absorbance detection.

2. Materials and methods

2.1. Reagents and chemicals

Standards of the analytes of interest were purchased from two different chemical companies: DHAA from Helix Biotech (Richmond, Canada) and abietic acid from Wako (Tokyo, Japan). Formic acid, HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Distilled and deionized water was used for the preparation of all solutions. The two mobile phases used for gradient elution were: (A) methanol–water (90:10, v/v) containing 0.06% (v/v) formic acid and (B) methanol–water (60:40, v/v) containing 0.1% (v/v) formic acid.

2.2. Standard preparation

The stock standards of DHAA and abietic acid were prepared from 10 mg of the respective acids dissolved in 10 ml of acetonitrile. A second stock solution containing each 10 μg/ml of DHAA and abietic acid were prepared by diluting the first solution with mobile phase A. Calibration curves were prepared by diluting the second stock solution to final concentrations of 0.01–1 μg/ml of DHAA and 0.025–1 μg/ml of abietic acid and with an injection volume of 50 μl.

2.3. Sample preparation

Twenty-nine bindi samples of different manufacturers were purchased in Singapore. Each piece of bindi sticker (10–20 mg) contains approximately 1–2 mg of adhesive. The SPE column used for sample cleaning was a 1-ml tube column containing 100 mg of C_{18} packing material (Supelco, USA). The column was preconditioned by rinsing with 1 ml of methanol and followed by 1 ml of water. One piece of bindi sticker was soaked in 1 ml of acetonitrile. After ultrasonication for 30 min, the sample was centrifuged at 1500 *g* for 2 min. An aliquot of 0.6 ml of sample was mixed with 0.6 ml of water. The mixture was centrifuged at 1500 *g* for 2 min and 1 ml of the supernatant was allowed to percolate through the preconditioned SPE column. The column was then washed with 1 ml of acetonitrile–water (60:40, v/v) and effluent was discarded. The analytes were then eluted with 1 ml of mobile phase A. An aliquot of 50 μl of the collected eluate was used for HPLC analysis. The results presented as observed are in μg/ml. After correction with a dilution factor of 2, the amount of DHAA and abietic acid contained in each bindi adhesive was calculated as μg per sample.

2.4. Chromatography

The HPLC system used consisted of a Waters Model 600E quaternary pumping system, a Model 996 photodiode array detector, a guard column connected to a Nova-Pak C_{18} analytical column (4 μm, 150 × 3.9 mm I.D.) and a Millennium 2010 software for peak identification and integration (Millipore, Milford, MA, USA). UV scans at peak apex were performed by the

present computerized system. The UV spectrum showed absorption maxima at 267 and 238 nm for DHAA and abietic acid, respectively. A Shimadzu Model RF-535 spectrofluorimetric detector (Kyoto, Japan) with slit width 15 nm was connected in series before the photodiode array detector. For DHAA determination, the excitation and emission wavelengths were set at 225 and 285 nm, respectively. Sample introduction was carried out using a Gilson autoinjector fitted with a 100-μl loop (Model 231-401; Villiers-le-Bel, France). The flow-rate was set at 1.0 ml/min with a gradient elution profile as follows: 0–0.8 min, 100% of mobile phase A; 1.2 min, A–B (50:50); 1.8–8 min, A–B (80:20) and 9–14 min, 100% A.

3. Results and discussion

3.1. Detection mode

HPLC determination of resin acids is generally carried out with UV absorbance detection [1,6–8]. In our initial experiment, DHAA and abietic acid were detected at 267 and 240 nm, respectively, by using a variable-wavelength detector (Model HP1050; Hewlett-Packard, Palo Alto, CA, USA). However, the detection of DHAA (0.5 μg/ml) at UV 267 nm was found to be rather insensitive (Fig. 1a). Using this detection method, most of the bindi samples containing less than 1 μg DHAA could not be detected. Therefore, a more sensitive and specific detection method by fluorimetry was developed. The maximum fluorescence of DHAA was obtained with excitation at 225 nm and emission at 285 nm. As shown in Fig. 1b, the peak height of DHAA was about 140 times higher than that measured with UV detection (Fig. 1a). The fluorimetric detection limit (signal-to-noise ratio 3) for DHAA was 0.01 μg/ml. On the other hand, a photodiode array detector was used for verification of abietic acid detection. It was found that the maximum UV absorbance was at 238 nm and the sensitivity is two times better than with the variable-wavelength detector. The lowest detection limit for abietic acid by using photodiode array detection was 0.025 μg/ml with a sample size of 50 μl.

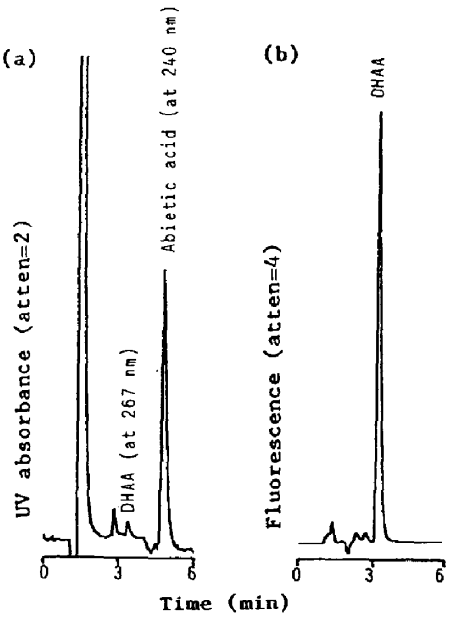

Fig. 1. Chromatograms for an injection of 50 μl of DHAA and abietic acid standards (0.5 μg/ml each) eluted with 100% of mobile phase A at 1.0 ml/min, and analyzed by (a) UV absorbance detection and (b) fluorimetric detection.

3.2. Efficiency of chromatographic analysis

DHAA and abietic acid are insoluble in water but readily dissolved in methanol or acetonitrile. For HPLC analysis, the chromatographic performances of the analytes were evaluated with different mobile phases in order to determine the optimum analytical condition. It was observed that the analytes were retained longer in a reversed-phase C_{18} column with 90% (v/v) methanol than with same concentration of acetonitrile. The retention time of these resin acids and other unknown components were also found to be susceptible to slight changes in the solvent concentration. However, the sensitivities of this effect among the analytes and components were not uniform. Furthermore, chromatographic performances of DHAA and abietic acid were poor if eluted with mobile phase containing less than 80% (v/v) methanol or 75% (v/v) acetonitrile.

Thus, separation of these resin acids in contact adhesive is usually difficult to achieve.

Nevertheless, it was found that addition of acetic or formic acid in 80% solvents in the mobile phase improved the chromatographic efficiency dramatically. Therefore, a gradient elution method using methanol-based solvent as the mobile phase with water and formic acid as modifier was developed. Using the present method, only two samples of the same manufacturer had DHAA closely eluted with interferences. The reliability of this method was verified by comparing the results obtained from the other 27 samples, with the same samples analyzed by using two other, different gradient profiles. The results showed close agreement among these three elution methods (data not shown) and the present method was considered the most efficient and time saving. In Fig. 2, chromatograms of a, b and c are a pure standard (0.5 μg/ml of DHAA and abietic acid), a blank and spiked sample (+ 0.5 μg/ml of DHAA and abietic acid), respectively. DHAA was determined by fluorimetric detection at 3.6 ($\pm$ 0.05) min. In Fig. 3, chromatograms of a, b and c are of the same injections of samples as in Fig. 2, abietic acid was detected at 6.1 ($\pm$ 0.05) min at UV 238 nm (photodiode array detection). The peaks were well separated with no spectroscopic evidence for coelution. Similar purity tests had been carried out for the rest of samples collected from different manufacturers, and results appeared promising. Only three samples had their abietic acid peaks interfered by adjacent peaks. The

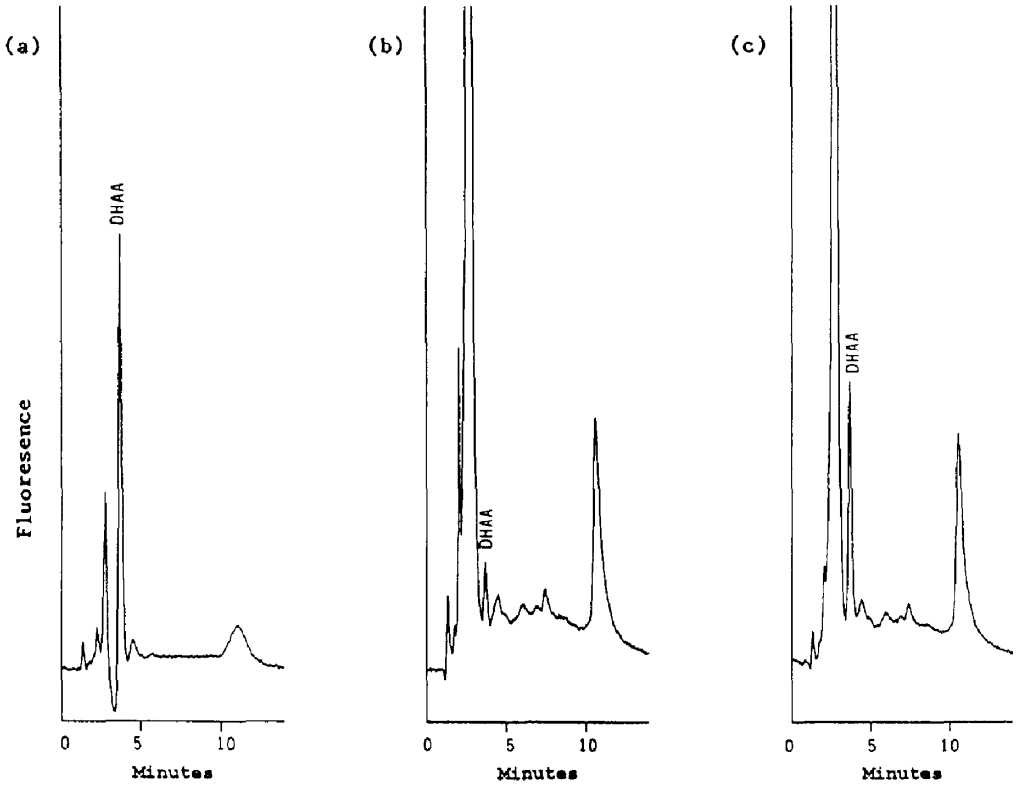

Fig. 2. Chromatograms of (a) a pure standard (0.5 μg/ml of DHAA and abietic acid), (b) a blank and (c) same sample supplemented with 0.5 μg/ml of DHAA and abietic acid, analyzed by the present gradient elution method with fluorimetric detection.

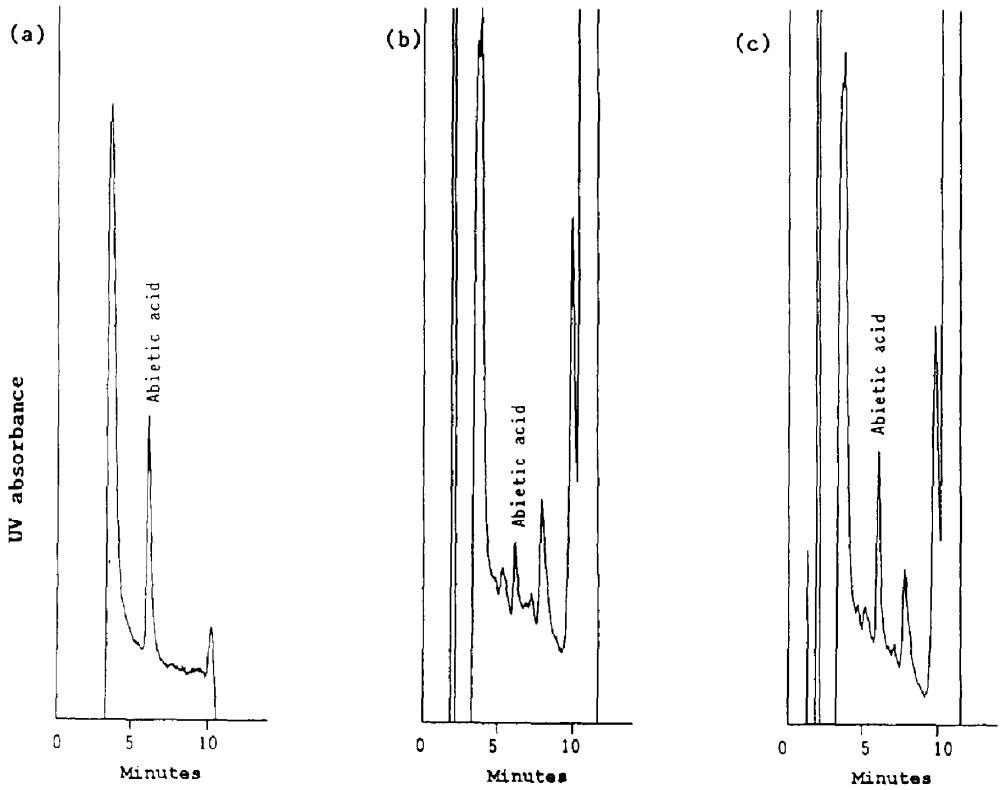

Fig. 3. Chromatograms of (a), (b) and (c) as in Fig. 2, but determined by photodiode array detection and reviewed at UV at 238 nm.

total analysis time was only 14 min per injection. Thus, the present HPLC method could be considered the most rapid, specific and reliable for identification and quantification of DHAA and abietic acid in bindi adhesive.

3.3. *Sample preparation, recovery and reproducibility*

Matrix interferences of the bindi samples varied from sample to sample due to different manufacturers. The HPLC determination of these resin acids was found to be rather complex either with UV or fluorimetric detection. Samples prepared by ultrasonication in dichloromethane, evaporation and reconstitution in methanol as suggested by Ehrin and Karlberg [7], showed serious interferences on HPLC analysis (data not shown). In our study, samples treated with acetonitrile alone were also difficult and non-specific due to void volume matrix interferences. Thus, the use of SPE for sample cleaning prior to HPLC analysis appears to be the most appropriate approach. The conditions and procedures had been worked out for maximum recovery. Pure standards with concentrations of 0.1, 0.2 and 0.5 μg/ml of DHAA and abietic acid, were processed with the same procedures as sample preparation and were used for determining the recovery, within-assay and day-to-day precision of the method. The mean recovery of various concentrations was 96% for DHAA and 102% for abietic acid (Table 1). The within-assay and day-to-day relative standard deviations

Table 1
Recovery and reproducibility ($n = 3$)

Sample (μg/ml)	Mean (μg/ml)	Recovery (%)	Relative standard deviation (%)	
			Within-day	Between-day
DHAA				
0.1	0.094	94	6.0	6.3
0.2	0.193	97	3.9	7.2
0.5	0.491	98	3.0	3.4
Mean		96	4.3	5.6
Abietic acid				
0.1	0.104	104	4.1	2.3
0.2	0.203	102	2.9	4.0
0.5	0.505	101	5.8	7.4
Mean		102	4.3	4.6

were generally less than 7% for both DHAA and abietic acid analysis ($n = 3$).

3.4. Linearity

Calibration was done by the external standard method. The calibration curves were linear for concentrations of DHAA in the range 0.01–1 μg/ml and 0.025–1 μg/ml for abietic acid. Typical regression equations and correlation coefficients (r) were $y = 102 + 1.6284 \cdot 10^4 x$ ($r = 0.99$) for DHAA and $y = 41 + 3.815 \cdot 10^3 x$ ($r = 0.99$) for abietic acid, where y is the peak height (μV) and x is the concentration of analytes (μg/ml). The relative standard deviations of slope and linearity of calibrations for between-day analysis ($n = 3$) were 0.6 and 0.08% for DHAA, and 3.4 and 0.08% for abietic acid; respectively.

3.5. Quantification of DHAA and abietic acid in bindi adhesive

DHAA and abietic acid are well known skin sensitizers. They can be found in various types of contact adhesives and facial cosmetics and can cause contact allergy [7–9]. In clinical practice, patients with contact dermatitis to these cosmetic items may be found to be sensitive to colophony on patch testing. The quantification of these allergens in actual contact items would be helpful for clinical diagnosis and management. Besides those samples that we were unable to measure due to interference or below detection limit, the mean value of DHAA for 26 samples was 0.36 (range 0.06–1.53) μg and that of abietic acid for 24 samples was 0.31 (range 0.05–0.81) μg per sample. The results demonstrate that DHAA and abietic acid are present in adhesives of most of the bindi samples tested in Singapore.

The proposed method was validated with 29 bindi samples available in Singapore. Approximately 1 mg of bindi adhesive containing as low as 0.002% of DHAA and 0.005% of abietic acid could be detected. The sensitivity of this proposed method for abietic acid analysis was 40 times higher than the earlier method [7]. An analytical run took only 14 min. The present method can be considered the most sensitive and reliable HPLC method for DHAA and abietic acid measurement in contact adhesives.

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