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Thin-layer chromatography and gas chromatography–mass spectrometry examination of shoe materials from patients with shoe dermatitis

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Shoe materials are known to be common causes of contact dermatitis, but the actual causative allergens are often unknown. When the clinical picture strongly suggests shoe dermatitis but the patient does not react to the screening series, one must extract and identify individual substances from the suspected shoes, and patch test with them to possibly prove the presence of a contact allergy (1–3).

Case 1

A 50-year-old male with shoe dermatitis developed symmetrical eczematous lesions on the dorsal and plantar

surfaces of the feet after wearing imitation leather sandals for 6 months (Fig. 1). His lesions healed when he was not wearing the sandals, but recurred after re-exposure.

Case 2

A 53-year-old female was diagnosed as having shoe dermatitis on the dorsal and plantar surfaces of the feet (Fig. 2). We also found atypical clinical features of shoe dermatitis, possibly caused by the herbal medication and antiseptic soap used to treat the recurrent symptoms of shoe dermatitis.

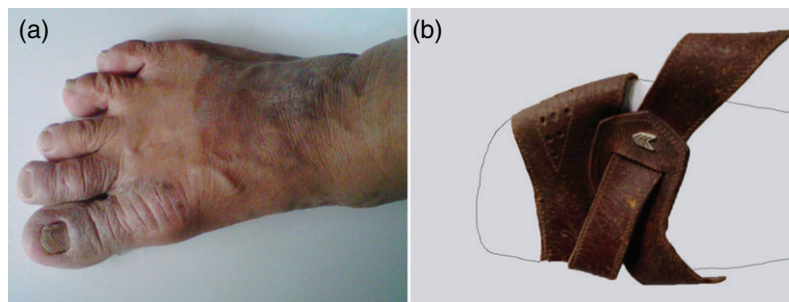


Fig. 1. (a) Eczematous lesions on the dorsum of the feet of patient 1. (b) Upper part of the sandal of patient 1, which is in direct contact with the skin lesions (b).

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Patch Testing

Both patients were patch tested with allergens from the European baseline series, the shoe series, and a series of 12 additional allergens, based on our own earlier studies of chemicals used in Indonesian leather and shoe factories (4). The results were negative.



Fig. 2. Eczematous lesions on the dorsum of the feet of patient 2.

Both patients were then patch tested with their suspected shoe materials (imitation leather) taken from the upper parts of the sandals, which were in direct contact with the corresponding areas of the skin lesion. Each patient was also patch tested with the material of the other patient. These materials were cut into pieces of area 1 cm² and thickness 2 mm, and were moistened with saline before being applied. Both patients reacted to the inner part of the sandal uppers of patient 1 (Table 1).

Extracts of suspected materials of both patients were prepared at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Indonesia by adding 80 ml of ethanol to 8 g of shoe material, extracting with an ultrasonic bath for 2 hr, and centrifuging for 5 min at 500 rpm. The supernatant was concentrated with a 'Buchi' vacuum evaporator (5). Both patients reacted to the extract of patient 1 but not to the extract of patient 2. Patch testing of both extracts in 10 healthy controls gave negative results.

Thin-layer chromatography (TLC) was performed in order to separate the components of the shoe extract of patient 1 and to obtain a TLC strip for patch testing (TLC plastic roll, Silica Gel 60F 254; Merck KGaA, Darmstadt, Germany). The TLC strip was prepared at the Department of Occupational and Environmental Dermatology,

Table 1. Test results from patch testing with the patch test series, shoe materials (patients 1 and 2), extracts (patients 1 and 2), and thin-layer chromatography (TLC) strip (made from the extract of patient 1)

Test material	Vehicle	Patient 1	Patient 2
Patient 1 shoe material (upper)	'as is'	+	+
Patient 2 shoe material	'as is'	–	–
Patient 1 shoe extract	Ethanol	+	+
Patient 2 shoe extract	Ethanol	–	–
Patient 1 TLC extract:			
Spot A	TLC strip	(+)	+
Spot B	TLC strip	(+)	+
Spot C	TLC strip	–	+
Spot D	TLC strip	–	+
2(3H)-Benzothiazolone	0.1% pet.	(+)	+
2(3H)-Benzothiazolone	1.0% pet.	(+)	+
Triclosan	2.0% pet.	–	–
European baseline series, including colophonium	–	–	–
Shoe series	–	–	–
Additional allergens (12 allergens)	–	–	–

Reactions: (+), doubtful reaction; +, non-vesicular reaction, palpable erythema, and slight infiltration.

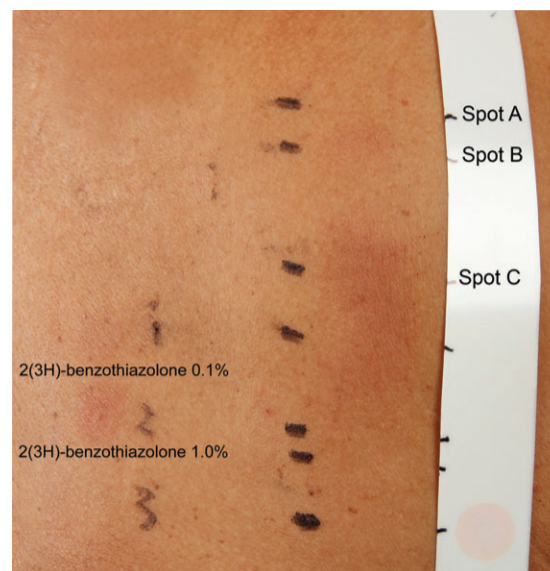


Fig. 3. Patient 2 reacted to spots A, B, C and D in the thin-layer chromatography strip, and to 2(3H)-benzothiazolone 0.1% pet. and 1.0% pet.

Malmö, Sweden. The TLC strip was removed after 48 hr, and read on D2, D4, and D7, according to ICDRG criteria (6). Both patients reacted to spots A and B on the TLC strip, and patient 2 also reacted to spots C and D (Fig. 3). Areas corresponding to the positive reactions were investigated by scraping off the silica gel for each spot from an

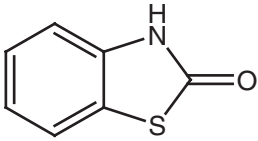
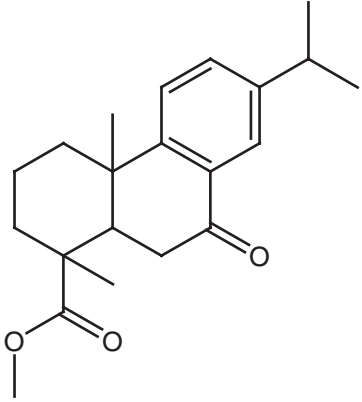
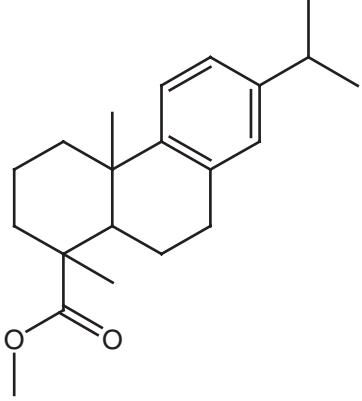
	2(3H)-Benzothiazolone CAS no. 934-34-9 MW 151
	7-Oxodehydroabietic acid methyl ester CAS no. 110936-78-2 MW 328
	Methyl dehydroabietate CAS no. 1235-74-1 MW 314

Fig. 4. Chemical structures, names, CAS numbers and molecular weights (MWs) of confirmed and suspected allergens.

exact copy of the tested TLC strip, and extracting the gel with a minimal amount of ethyl acetate. The resulting solutions were analysed by gas chromatography–mass spectrometry (GCMS) (7). It was found that the positive TLC spots contained methyl dehydroabietate (spot A), 7-oxodehydroabietic acid methyl ester (spot B), and 2(3H)-benzothiazolone (spot C) (Figs. 3 and 4). The main component in spot D was unidentifiable. Both patients were patch tested with 2(3H)-benzothiazolone at two concentrations, 0.1% and 1.0% in pet. Patient 1 showed a doubtful reaction, and patient 2 was found to be positive to both concentrations of 2(3H)-benzothiazolone (Fig. 3).

Discussion

To improve accuracy, patch testing with the patient's own products is often recommended. However, in a product, an allergen may be present at a concentration so low that it gives a negative patch test result. When patch testing with an extract of the product, the chemical components of a product will be patch tested at a higher concentration, and the patch test will therefore be more sensitive and also adjust for the repeated exposure to a product that is often needed to cause allergic eczema (5). With regard to shoes, further investigation is often needed in order to identify the allergen. Patch testing with TLC strips of

Table 2. Substances in the extracts of the shoe materials of both patients analysed by gas chromatography–mass spectrometry

Patient	Substances
Patient 1	Cinnamic acid (CAS no. 140-10-3) 2(3H)-Benzothiazolone (CAS no. 934-34-9) Triclosan (CAS no. 3380-34-5) Colophonium Diethylhexyl phthalate (CAS no. 117-81-7) Diisobutyl phthalate (CAS no. 84-69-5) 2,4-Di-tert-butylphenol (CAS no. 96-76-4) 1-Dodecanol
Patient 2	Cinnamic acid (CAS no. 140-10-3) Diisobutyl phthalate (CAS no. 84-69-5) Diethylhexyl phthalate (CAS no. 117-81-7) Nonylphenol ethoxylates 1-Dodecanol 1-Chlordodecan

the positive extract is often helpful for identification of allergens (6). The chemical substances in the extract are separated on the TLC strip, and this greatly facilitates the chemical analysis. Identification of the substances present in a positive TLC strip patch can often be performed with GCMS (or a similar method).

As both patients came to our clinic at the same time, the shoe materials of each were patch tested on both. The shoe material and extract of patient 1 were positive in both patients, whereas the shoe material and extract of patient 2 were negative in both. Further TLC testing with TLC from shoe extract of patient 1 gave positive reactions in patient 2 (Table 1).

Because the extracts were to be sent to an external laboratory and the clinical investigation was to be performed in a geographically remote area, both extracts were actually analysed before patch testing (Table 2). We patch tested 2(3H)-benzothiazolone (CAS no. 934-34-9) and the preservative triclosan in both patients. Triclosan gave negative results in both patients, but patient 2 reacted positively to 2(3H)-benzothiazolone, which (according to the GCMS analysis) was present in the shoe extract of patient 1.

References

- Lynde C W, Warshawski L, Mitchell J C. Patch test results with a shoe wear screening tray in 119 patients, 1977–1980. *Contact Dermatitis* 1982; **8**: 423–425.
- Angelini G, Vena G A, Meneghini C L. Shoe contact dermatitis. *Contact Dermatitis* 1980; **6**: 279–283.
- Romaguera C. Shoe contact dermatitis. *Int J Dermatol* 1987; **26**: 532–535.
- Febriana S A, Jungbauer F, Soebono H, Coenraads P J. Inventory of the chemicals and the exposure of the workers' skin to these at two leather factories in Indonesia. *Int Arch Occup Environ Health* 2012; **85**: 517–526.
- Bruze M. The use of ultrasonic bath extracts in the diagnosis of contact allergy and allergic contact dermatitis. In: *Patch Testing Tips*, Lachapelle JM, Bruze M, Elsner P (eds): Berlin, Heidelberg, Springer-Verlag, 2014: pp. 129–142.
- Bruze M, Frick M, Persson L. Patch testing with thin-layer chromatograms. *Contact Dermatitis* 2003; **48**: 278–279.
- Svedman C, Zimerson E, Bruze M. Allergic contact dermatitis caused by benzanthrone in a pair of trousers. *Contact Dermatitis* 2014; **71**: 54–57.
- Mathur A K, Khanna S K. Dermal toxicity due to industrial chemicals. *Skin Pharmacol Appl Skin Physiol* 2002; **15**: 147–153.

Patch testing with the TLC strip showed that patient 2 reacted to four spots on the TLC strip (spots A, B, C, and D). 2(3H)-Benzothiazolone was identified in spot C. In spots A and B, respectively, methyl dehydroabietate and 7-oxodehydroabietic acid methyl ester were found; both of these are derivatives of colophonium. The main component in spot D was unidentifiable, and was therefore not patch tested. Also, spot C contained some substances that could not be identified.

Patient 1 reacted only to his own shoe material and extract. Spot C, with 2(3H)-benzothiazolone, was negative, patch testing with this allergen gave doubtful results, as were the spots containing colophonium derivatives (spots A and B).

Patient 2 did not react to either her own shoe material or extract, but she did react to the shoe material and extract of patient 1 and the allergens found there. This indirectly emphasizes the need for an updated shoe series; allergens giving positive reactions may not be common, but could very well be present in shoes. 2(3H)-Benzothiazolone is a rare allergen that we have not previously encountered. However, an Indian paper reported it as a common sensitizer used as a corrosion inhibitor (8). This substance could have been added to the product, or it could be a degradation product of the rubber chemical mercaptobenzothiazole or an oxidation product of benzothiazole. We previously discovered the latter substance as an antimicrobial agent used in bras (unpublished findings).

Methyl dehydroabietate and 7-oxodehydroabietic acid methyl ester indicate the presence of colophonium (colophony, rosin) or modified colophonium in shoe materials, probably as a component of glue. As colophonium can be produced either by pines or by other plants, it may contain different allergens. The best approach is to patch test the colophony to which the patient was exposed; however, this was not possible in this case, as the two detected colophony substances were not available at the time. At least with the information from the TLC patch testing, patient 2 can be advised to not wear shoes that possibly contain colophonium-derived glues.