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Original Paper

SPE and HPLC/UV of resin acids in colophonium-containing products

A new method, involving SPE and HPLC/UV diode-array detection (DAD), was developed for the quantification of colophonium components in different consumer products, such as cosmetics. Colophonium is a common cause of contact dermatitis since its components can oxidize into allergens on exposure to air. Three different resin acids were used as markers for native and oxidized colophonium, abietic acid (AbA), dehydroabietic acid (DeA), and 7-oxodehydroabietic acid (7-O-DeA). The SPE method, utilizing a mixed-mode hydrophobic and anion exchange retention mechanism, was shown to yield very clean extracts. The use of a urea-embedded C₁₂ HPLC stationary phase improved the separation of the resin acids compared to common C₁₈. Concentrations higher than 2 mg/g of both AbA and DeA were detected in wax strips. In this product also 7-O-DeA, a marker for oxidized colophonium, was detected at a level of 28 µg/g. The LODs were in the range of 7–19 µg/g and the LOQs 22–56 µg/g. The method is simple to use and can be applied on many types of technical products, not only cosmetics. For the first time, a method for technical products was developed, which separates AbA from pimaric acid.

Keywords: Allergic contact dermatitis / Colophonium / HPLC/UV / Resin acids / SPE

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1 Introduction

Colophonium (rosin) is a common cause of contact allergy and allergic contact dermatitis (ACD) [1]. There are three types of rosin depending on the ways of recovery – gum rosin, wood rosin, and tall oil rosin. Gum rosin is obtained from living trees, wood rosin is obtained from old pine stumps, and tall oil rosin is obtained as a by-product in the sulfate pulping of coniferous trees. The different types are used interchangeably and most often chemically modified. In this paper we use the term colophonium, which most often is synonymous with gum rosin, but also can be used for all three types of rosin, especially in the dermatological literature. Colophonium has valuable properties for many types of applications as it is a very good adhesive and emulgator. The largest single consumption is in the sizing of paper and

paperboard where it is added after modification to increase water resistance. Consequently, ACD caused by colophonium in different commercial and technical products, such as paper, printing ink, adhesives, household and beauty products is reported in the literature. For instance, colophonium has been detected in mascaras marketed as “hypoallergenic” and the content in the products investigated was enough to cause elicitations in sensitized individuals [2]. Extensive reviews on the usage of colophonium have been presented during the years [3–6].

In clinical studies, significant concomitant positive patch test reactions have been observed involving colophonium and reactions to fragrance markers (fragrance mix and balsam of Peru) among consecutive patients [7–9]. The test reactions described are not only due to cross-reactions to the same compounds present in the tested materials, but are best explained as the result of concomitant sensitization caused by simultaneous exposure to a wide variety of fragrance chemicals and colophonium components in products in everyday life [10].

Colophonium contains a complex mixture of resin acids (about 90%) and neutral substances (10%) which varies depending on species, recovery process, and storage condition. The major acids are abietic acid (AbA) and dehydroabietic acid (DeA) (Fig. 1) [1]. Oxidized material is

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Abbreviations: AbA, abietic acid; ACD, allergic contact dermatitis; DAD, diode-array detection/detector; DeA, dehydroabietic acid; EPA, *cis*-5,8,11,14,17-eicosapentaenoic acid; IS, internal standard; 7-O-DeA, 7-oxodehydroabietic acid

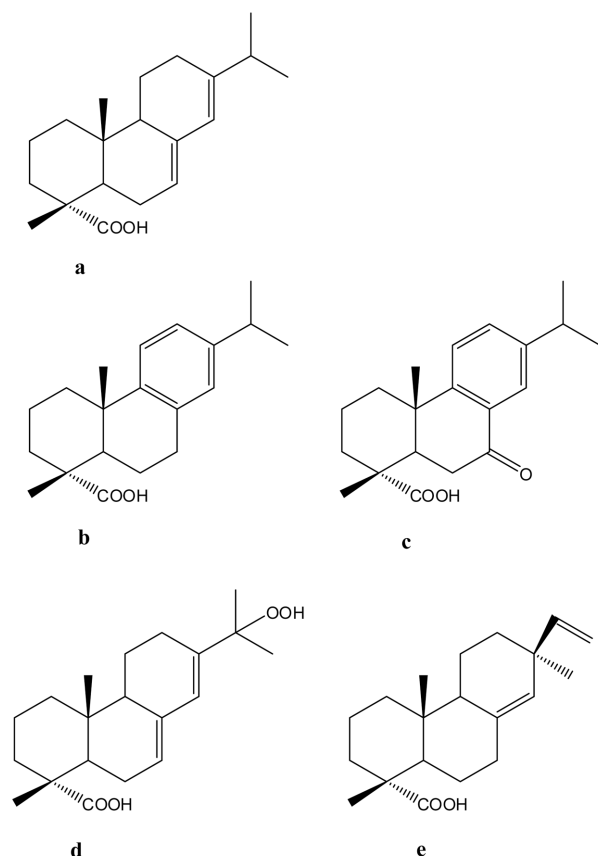


Figure 1. Chemical structures of the markers of colophonium. (a) AbA, (b) DeA, (c) 7-O-DeA. Also the structures of the major allergen (d) 15-hydroperoxyabietic acid, and of (e) pimaric acid are shown.

present in colophonium due to air exposure during normal handling and storage. Extensive studies have identified that oxidized compounds formed at air exposure originate mainly from AbA [3, 11–14]. While AbA itself is a weak allergen or nonallergenic, some of the oxidation products formed are allergens. One of the most prominent oxidation products is 15-hydroperoxyabietic acid (Fig. 1), which is a potent allergen [10, 11, 15].

The objective of the present work was to develop a suitable HPLC method with UV diode-array detection (DAD) to be used for screening with regard to the content of colophonium in different products. Traditionally, the major resin acids in colophonium are quantified using GC/FID [16–20] or GC/MS [21–23]. These methods have high separation efficiency, but require derivatization before analysis. As the method should be used for screening purposes it is important that it is a robust, inexpensive and simple method, preferably not involving a time-consuming derivatization step or highly dangerous materials. Furthermore, it is important to be able to quantify not only the major components AbA and DeA, but also a marker for oxidized products, that are the major aller-

gens in colophonium. HPLC methods, with either UV [19, 24–27] or fluorescence [26, 27] have been described previously for the analysis of different technical products and food samples, but neither of those has included the quantification of any of the oxidized resin acids. Analyses based only on the nonoxidized terpenoic acids will not give any information on the content of allergenic oxidized resin acids. A small detected amount of AbA might be due to a small amount of colophonium present, but could also be due to an extensive oxidation. We chose the oxidation product 7-oxodehydroabietic acid (7-O-DeA) (Fig. 1) as a marker for oxidized colophonium. This oxidation product is stable enough to be used as a reference compound and not as allergenic as 15-hydroperoxyabietic acid [28]. 7-O-DeA was recently quantified using LC/MS in ESI mode, in the measurement of dermal exposure to resin acids [29].

HPLC methods using conventional C_{18} or C_8 stationary phases have been shown to exhibit insufficient selectivity to separate the major resin acids in colophonium. For instance, chromatographic separation of pimaric acid (Fig. 1) and AbA has not been accomplished using a C_{18} column. A urea-embedded column was therefore chosen in the present work. The added selectivity is gained by the polar urea function embedded between the spacer and a C_{12} chain. The urea may interact with the resin acids by ionic interactions or hydrogen bonding, and yields a better wettability of the alkyl chains. The latter could contribute to a higher shape-selectivity for the compounds compared to conventional RP columns.

For clean-up of the complex samples, SPE was evaluated using a mixed-mode anion-exchanger in order to gain selectivity for the lipophilic resin acids. Similar to the HPLC phase, this SPE phase also combines different retention mechanisms, *i.e.*, hydrophobic interaction with polymeric divinylbenzene units and anion exchange with quaternary amine groups.

2 Experimental

2.1 Chemicals

AbA, DeA, and pimaric acid (all >95% purity) were obtained from Helix Biotech (Vancouver, Canada). *cis*-5,8,11,14,17-Eicosapentaenoic acid (EPA, >98.5%) used as an internal standard (IS) was purchased from Fluka (Sigma-Aldrich Chemie, Steinheim, Germany). 7-O-DeA was synthesized as described below. All compounds were stored in a freezer.

Standard stock solutions of each compound were prepared in 100% of ACN at a concentration of 1 mg/mL. They were stored in a freezer throughout the study.

Standard solutions for HPLC were made in ACN/Milli-Q water 9:1 v/v with 0.2% formic acid. They were stored in a refrigerator and were stable for at least 2 wk (not tested

for longer periods). The purity of the standard solutions was checked by HPLC analysis each day. The relative absorption ratios did not change during the time the standards were used.

Methanol of 99.8% purity (Hipersolv, BDH Prolabo) was purchased from VWR International (Leicestershire, UK), formic acid of 98–100% purity from Scharlau Chemie S.A. (Barcelona, Spain) and ACN of Chromasolv quality from Sigma–Aldrich.

Gum rosin (colophonium) samples (unmodified and maleic anhydride-modified from Soccer, Portugal) and unmodified colophonium (from Fluka) were dissolved in 100% ACN to approximately 1.5 mg/mL.

Cosmetic product samples of different brands, lip gloss, depilatory wax strips, and skin foundation were analyzed with respect to the target resin acids. According to the manufacturer the depilatory wax contained triethylene glycol rosinate and glyceryl rosinate (concentrations not declared). A foundation from Face (Stockholm, Sweden), containing no detectable amounts of resin acids, was spiked with standard compounds at different concentration levels, from 1 up to 500 µg/g of each compound, in order to evaluate recoveries, LODs, linearity, and precision.

2.2 Synthesis of 7-O-DeA

The 7-O-DeA was synthesized in two steps, where the first step involves an optimized procedure from Fleck *et al.* [30].

A mixture of AbA (4.16 g, 13.75 mmol) and 6% Pd/C (0.26 g, 6% w/w) was heated to 245°C for 1 h under nitrogen in a two-necked round bottom flask. After cooling, the solid was dissolved with ethyl acetate (4 × 50 mL, or until all was dissolved) and filtered through celite. The organic layer was then extracted with 0.25 M sodium hydroxide (2 × 100 mL) followed by acidifying the aqueous layer with 1 M hydrochloric acid. The crude product was obtained by washing the aqueous layer with ethyl acetate (3 × 50 mL), drying the organic phase with magnesium sulfate and finally concentrated under reduced pressure. Purification by flash chromatography (ethyl acetate/hexane/TFA 14:85:1) yielded 2.50 g (58%) DeA. This was in agreement with previously reported data from Ayer *et al.* [31].

To a mixture of DeA (2.10 g, 6.99 mmol) and potassium hydroxide (0.40 g) in water (40 mL) was added a solution of potassium permanganate (2.80 g, 17.72 mmol) in water (60 mL) drop-wise at room temperature. The resulting mixture was stirred for 2.5 h followed by saturation with sulfur dioxide. A white precipitate was formed and filtered off, washed with water, and air-dried over night. Purification by flash chromatography (ethylacetate/hexane/TFA 14:85:1) yielded 1.15 g (52%) 7-O-DeA of 95%

purity according to NMR. This was in agreement with previously reported data from Ayer *et al.* [31].

2.3 Equipment

An Agilent 1100 gradient system was used for HPLC analyses, equipped with an injection loop of 20 µL, a DAD and a column heater set at 25°C. Higher temperatures up to 50°C were tested to decrease peak broadening, but were not useful. The HPLC column was a Prism RP-12 from Thermo Fisher Scientific (4.6 mm id, length 150 mm, particle size 3 µm) with a urea-embedded C₁₂ stationary phase. For SPE, Oasis MAX columns (6 mL, 500 mg) from Waters were used.

2.4 Ultrasonic extraction from beauty products

Weighed samples of 1 g were sonicated in 20 mL of ACN during 30 min. An aliquot of 500 µL EPA from a 928 µg/mL solution, was added as an IS prior to extraction.

For all samples except depilatory wax strips, the extract was transferred to plastic tubes and centrifuged during 15 min at 4000 rpm. The supernatant liquid was transferred to a flask, while the pellet was re-dissolved in ACN, ultrasonicated, and centrifuged using the same procedure. The supernatant liquids were pooled in the flask and evaporated in a rotavapor to a volume of approximately 5 mL. Adjustment of the volume to 7 mL was made by adding ACN. Milli-Q water was then added to a final volume of 10 mL containing ACN/water 7:3. Addition of water made some of the solutions opaque. In those cases, filtration through a syringe filter (0.45 µm polypropylene membrane) was performed prior to the SPE.

For depilatory wax strips, the sample was evaporated using rotavapor to about 5 mL directly after ultrasonic extraction. Adjustment of the volume to 7 mL was made by adding ACN. Milli-Q water was then added to a final volume of 10 mL containing ACN/water 7:3. The sample was transferred to plastic tubes and centrifuged at 4000 rpm during 15 min. The supernatant liquid was loaded directly on the conditioned SPE, without syringe filtration.

2.5 SPE

The SPE stationary phase was conditioned by percolating (i) 3 mL of methanol and (ii) 3 mL of Milli-Q water through the SPE column. The sample was loaded and the column was then washed with (i) 3 mL of 50 mM sodium acetate in Milli-Q water to eliminate very polar neutrals or bases, (ii) 4 mL of methanol to wash out lipophilic neutrals and finally (iii) 1 mL of 2% formic acid in methanol. The sample was then eluted with 2 mL of 2% formic acid in methanol. An additional volume of 1 mL 2% formic

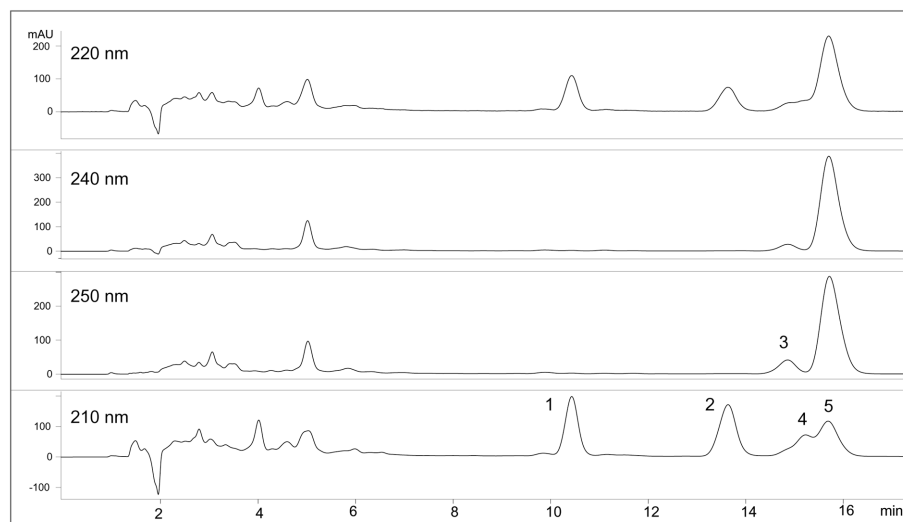


Figure 2. HPLC/UV-DAD chromatograms of a gum rosin sample at different wavelengths. (1) DeA, (2) EPA (IS), (3) unknown, (4) pimaric acid, and (5) AbA. 7-O-DeA was not detected. For chromatographic conditions, see Section 2.

acid in methanol was gathered in case of breakthrough. Fresh formic acid solution was made daily.

2.6 Analysis of gum rosin

EPA, 200 μL (186 μg) was added as IS to 2 mL of 1.5 mg/mL gum rosin solutions. The gum rosin samples were analyzed directly using HPLC after syringe filtration (without SPE clean-up).

2.7 HPLC/UV-DAD method

Methanol/water 8:2 with 0.05% formic acid was used isocratically as mobile phase at a flow rate of 1 mL/min (yielding a pressure of approx 240 bar). Four different wavelengths were used for optimal sensitivity/selectivity: 210, 220, 240, and 250 nm. The different absorption ratios were used for identification of the compounds. The amount of formic acid was found to be critical for both retention and noise level.

A calibration curve was made from injected concentrations in the interval 0.8–500 ng/ μL of each compound. For quantification, a single-point calibration was performed by a daily triplicate injection of a 250 ng/ μL standard. Quantification was performed using relative response factors of analyte/IS.

Specific absorption ratios (CVs below 10%) were used for identification of the different compounds: 1.4 for AbA (240 nm/250 nm), 0.55 for DeA (220 nm/210 nm), 0.53, 0.26, and 0.45 for 7-O-DeA (220 nm/210 nm, 240 nm/210 nm, and 250 nm/210 nm, respectively), 0.40 for EPA (220 nm/210 nm). It should be noted, however, that the absorption ratios to some extent may be dependent on the specific detector used.

Quantification of DeA and EPA was performed by integration of peaks at 210 nm. For both AbA and 7-O-DeA integration was made at 240 nm.

3 Results

3.1 Selectivity of the HPLC method and accuracy of quantification

The HPLC method developed is able to separate and identify the reference components AbA and DeA in gum rosin samples containing a large number of interfering resin acids. A chromatogram from an unmodified gum rosin sample is shown in Fig. 2. Pimaric acid at 15.2 min co-elutes with another compound (not identified) at 14.8 min. By using the wavelength at 240 nm, integration of the AbA peak could be performed without interference from the pimaric acid peak. The absorption ratios were 1.35 for AbA (240 nm/250 nm), 0.54 for DeA (220 nm/210 nm) and 0.43 for IS (220 nm/210 nm) ($n = 3$, CV <5%). The values are in agreement with those obtained for the standard (see Section 2). The oxidized compound, 7-O-DeA, could not be detected in any of the rosin samples. The concentrations of AbA and DeA were 26 (by weight) and 6%, respectively, in unmodified gum rosin from Soccer (CV <5%, $n = 3$). In unmodified gum rosin from Fluka the levels were 69 and 4% (CV <5%, $n = 3$), respectively. Rosin modified with maleic anhydride showed levels of unreacted AbA and DeA at 7 and 2% (CV 10%), respectively. Concentrations of 40 and 5% (by weight), respectively, for unmodified gum rosin has been reported previously by Karlberg *et al.* [32].

With the chosen mobile phase and flow rate, the selected resin acids in colophonium could be separated within 20 min. It was important to control the formic

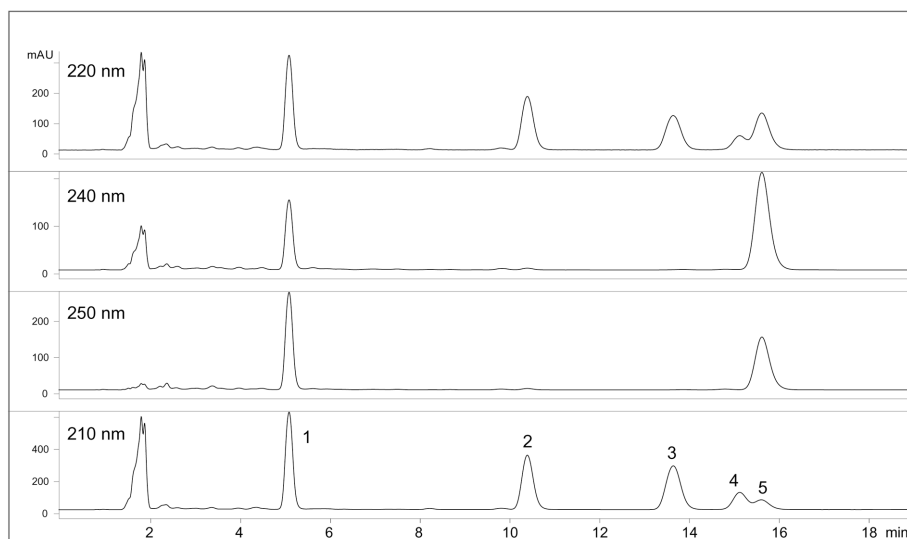


Figure 3. HPLC/UV-DAD chromatograms of a spiked foundation sample (500 ng/g of each compound) after SPE. (1) 7-*O*-DeA, (2) DeA, (3) EPA (IS), (4) pimaric acid, and (5) AbA. For chromatographic conditions, see Section 2.

acid concentration in the mobile phase thoroughly. Too low a concentration (0.02%) deteriorated the separation and too high a level (0.1%) increased the noise severely. Isocratic elution is to be preferred, although this leads to peak broadening of especially AbA. A solvent gradient was shown to increase the noise and LODs extensively.

3.2 Method evaluation with spiked skin foundation samples

The repeatability (within-day variation) in terms of CVs, recoveries and LODs were determined at two levels based on standard addition to a blank foundation sample without detectable amounts of resin acids. When resin acids in a fatty matrix is subjected to SPE on the MAX Oasis column, the neutrals and very lipophilic compounds are eliminated from the SPE with pure methanol, while the resin acids are eluted and enriched with acidic methanol, interrupting both the hydrophobic and anionic interactions with the stationary phase. The result is a very clean chromatogram as shown for a spiked sample in Fig. 3. As in the case of colophonium samples, the absorption ratios were in agreement with the standard values. The values for 7-*O*-DeA were 0.51, 0.24, and 0.44 (220 nm/210 nm, 240 nm/210 nm, and 250 nm/210 nm, respectively). The results from the evaluation at low levels (50–90 µg/g of each compound) are shown in Table 1. The recoveries from SPE were estimated using EPA as volumetric IS (recovery of EPA 97%, $n = 3$, tested with pimaric acid as volumetric IS). The method LOD, in µg/g of spiked sample, was calculated as three times the SD ($n = 6$). The method LOQ was calculated based on the spiked cosmetic samples using the equation: $LOQ = \text{blank} + 3 \times LOD$. This was equal to $LOQ = 3 \times LOD$ since there was no detectable blank values. The results from standard addition at higher levels are shown in Table 2.

Table 1. Evaluation of SPE from a foundation sample spiked at low levels, 50–90 µg/g. CV in %, $n = 6$. LOD and LOQ values are in µg/g.

Compound	CV%	% Recovery	LOD	LOQ
AbA	3	85	7	22
DeA	13	84	19	58
7- <i>O</i> -DeA	11	91	13	39

Table 2. Evaluation of SPE from a foundation sample spiked at high levels, 135–250 µg/g. CV in %, $n = 6$.

Compound	CV%	% Recovery
AbA	7	94
DeA	5	92
7- <i>O</i> -DeA	8	94

The linearity was tested by multiple analyses ($n = 10$). The method was found to be linear in the concentration range of approximately 1–400 µg/g (r^2 : 0.999–1.000).

3.3 Resin acids in cosmetic products

Different common cosmetic products, lip gloss, skin foundation, and depilatory wax strips (for body and face, respectively) were investigated with regard to their content of resin acids. The target compounds could be quantified in only one of the products, body depilatory wax strips. The concentrations were found to be 2301, 2517, and 27.8 µg/g for AbA, DeA, and 7-*O*-DeA, respectively. (CVs: 8, 12, and 17%, respectively). The relative concentrations of AbA and 7-*O*-DeA indicate that at least 1% of AbA in the wax had oxidized. For the other products, traces of AbA and DeA could be observed, however, the concentrations were below LODs. Pimaric acid was also detected, but was not quantified.

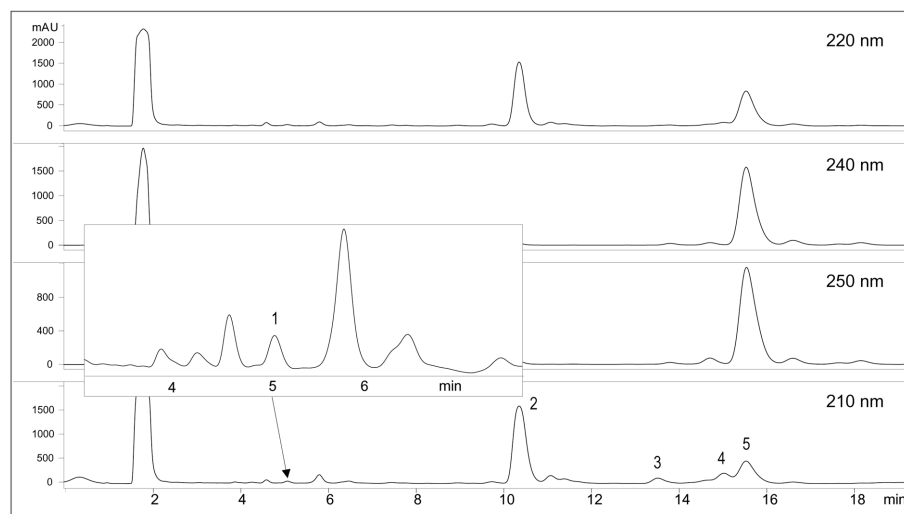


Figure 4. HPLC/UV-DAD chromatograms of resin acids extracted from 1 g of depilatory wax. (1) 7-*O*-DeA, (2) DeA, (3) EPA (IS), (4) unknown, and (5) AbA.

In Fig. 4, a chromatogram of a depilatory wax sample is shown. As in the case of the spiked samples, the SPE method efficiently removed interferences, resulting in a clean chromatogram.

4 Discussion

A new methodology for simple detection and quantification of the resin acids AbA and DeA in unmodified colophonium (rosin) was developed. For the first time, AbA and pimaric acid can be detected separately in technical products using HPLC, thanks to a polar-embedded stationary phase with high shape selectivity. This new method also includes the possibility of detection and quantification of one of the major oxidation products, 7-*O*-DeA, obtained at the oxidative degradation of colophonium in contact with air. High selectivity is needed due to the complexity of the products to be analyzed. This is obtained by a DAD, which allows selectivity without using MS.

For correct quantification it is highly recommended to daily check the purity of the standard solutions, since the resin acids, especially AbA, are readily degraded by oxidation. In this study it was found that the reference solutions were stable for at least 14 days if stored in the refrigerator. The solid compounds and stock solutions should be stored in a freezer. The purity of the standards can be checked in the HPLC analysis and by comparing the relative absorption ratios analyte/IS at each wavelength.

According to the EU legislation on dangerous substances – Annex 1 (Directive 67/548 /EEC) a content of >1% colophonium in a product must be declared and the product must be labeled with risk phrase R 43 (“May cause sensitization by skin contact”). However, colophonium is a mixture of many compounds. Quantifying a mixture used as an ingredient of another mixture cannot

be achieved by any analytical means. This applies to any natural extracts used as ingredient of a compounded consumer product. To overcome the impossibility of quantifying a complex ingredient in a product, the quantification of tracers (= defined substances) specific of the complex natural sources is feasible. Therefore, quantification must be based on chemical analyses of specific major compounds. For colophonium we have chosen to quantify the two major resin acids. However, it is not possible to determine the exact content of colophonium, since there is a difference in the acid concentrations due to variations in extraction, handling, storage, and manufacturing of various types of colophonium (rosin) which is most pronounced for DeA. The content of this resin acid can be 5–10% in gum rosin and around 30% in tall oil rosin. For AbA the variation is smaller, the concentration is normally between 30 and 50% for gum rosin and 35 and 40% for tall oil rosin. Thus, when estimating the colophonium content, quantification of AbA rather than DeA is to be preferred from this perspective.

A low level of AbA may indicate a low level of colophonium. However, it is important to check that the low level is not due to an oxidative decomposition of AbA, resulting in highly allergenic compounds such as 15-hydroperoxyabiatic acid. To avoid such a misinterpretation, we also used DeA as a marker, a much more stable compound than AbA, and 7-*O*-DeA as a tracer for the oxidation of AbA. High values of these tracers together with a low level of AbA indicate that AbA has decomposed extensively. In such a case, the content of colophonium is larger than estimation based on only AbA determination as the presence of allergenic oxidation products might be high.

In general, two major types of analyses for colophonium components are described in the literature. Traditionally, GC/FID methods with derivatization to achieve a high separation efficiency have been used for instance

in the pulp industry [16, 17]. Later on, LC methods with UV, fluorescence, and MS have been developed, most often for the analysis of specific products [24–27]. The method presented in this work is not restricted to a specific group of consumer products. Rather, it can be used universally, only limited by the methods of sample preparations.

In the present investigation the SPE phase used for sample preparation is a mixture of lipophilic and weak anion exchanging sites, which showed a high selectivity toward the resin acids. Other lipophilic compounds can be washed out from the column by using methanol, while the resin acids are retained until acid is added. Very clean extracts were obtained from all investigated products after the SPE step, according to the LC analyses.

Only a wax strip for hair removal contained significant concentrations of colophonium. For the other products (lip gloss, foundation, wax strips for face) the levels were below LODs. The wax strips were declared to contain triethylene glycol and glyceryl modified rosin, but a part of the rosin was left unmodified, since AbA, DeA, and 7-O-DeA could be detected. Commercial modifications are usually interrupted when the desired technical properties are obtained leaving some colophonium unmodified. Thus, colophonium components can cause ACD also from products claimed not to contain unmodified colophonium [5]. Many cases of ACD caused by colophonium components present in modified rosin are reported in the literature, e.g., from adhesives in shoes [33].

5 Concluding remarks

Earlier published analytical methods for identification and quantification of the major colophonium components have been thoroughly scrutinized from different aspects, such as the possibility to be used outside very sophisticated analytical laboratories, robustness, and possibility to detect compounds without derivatization. Based on this, it was concluded that a new method was needed for the purpose of the present study. We have thus developed an RP HPLC method with UV DAD. The method is robust, simple to use, without using toxic chemicals and has a high specificity compared to ordinary UV methods. Pure nonoxidized resin acids with a stable major oxidation product, as a marker for a possible autoxidation and the formation of allergenic oxidation products, have been used as reference substances.

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The authors declared no conflict of interest.

6 References

- [1] Karlberg, A.-T., Colophony, in: Kanerva, L., Elsner, P., Wahlberg, J. E., Maibach, H. I. (Eds), *Handbook of Occupational Dermatology*, Springer-Verlag, Heidelberg 2000, pp. 509–516.
- [2] Karlberg, A.-T., Lidén, C., Ehrin, E., *Acta Derm. Venereol.* 1991, 71, 445–447.
- [3] Karlberg, A.-T., *Acta Derm. Venereol.* 1988, 139, 1–43.
- [4] Downs, A. M. R., Sansom, J. E., *Contact Derm.* 1999, 41, 305–310.
- [5] Gäfvert, E., *Acta Derm. Venereol.* 1994, 184, 1–36.
- [6] Färm, G., *Acta Derm. Venereol.* 1997, 201, 1–42.
- [7] Färm, G., *Contact Derm.* 1996, 34, 93–100.
- [8] Wohrl, S., Hemmer, W., Focke, M., Gotz, M., Jarisch, R., *Br. J. Dermatol.* 2001, 145, 268–273.
- [9] Matura, M., Sköld, M., Börje, A., Andersen, K. E., Bruze, M., Frosch, P., Goossens, A., Johansen, J. D., Svedman, C., White, I. R., Karlberg, A.-T., *Contact Derm.* 2005, 52, 320–328.
- [10] Bråred-Christensson, J., Matura, M., Backtorp, C., Borje, A., Nilsson, J. L. G., Karlberg, A.-T., *Contact Derm.* 2006, 55, 230–237.
- [11] Karlberg, A.-T., Bohlinger, K., Boman, A., Hacksell, U., Hermansson, J., Jacobsson, S., Nilsson, J. L. G., *J. Pharm. Pharmacol.* 1988, 40, 42–47.
- [12] Karlberg, A.-T., *Am. J. Contact Derm.* 1991, 2, 43–49.
- [13] Hausen, B. M., Boerries, M., Budianto, E., Krohn, K., *Contact Derm.* 1993, 29, 234–240.
- [14] Gäfvert, E., Shao, L. P., Karlberg, A. T., Nilsson, U., Nilsson, J. L., *Chem. Res. Toxicol.* 1994, 7, 260–266.
- [15] Karlberg, A.-T., Gäfvert, E., *Contact Derm.* 1996, 35, 201–207.
- [16] Holmbom, B., *J. Am. Oil Chem. Soc.* 1977, 54, 289–293.
- [17] Örså, F., Holmbom, B., *J. Pulp Pap. Sci.* 1994, 20, 361–365.
- [18] Karlberg, A. T., Magnusson, K., *Contact Derm.* 1996, 34, 176–80.
- [19] Karlberg, A. T., Gäfvert, E., Meding, B., Stenberg, B., *Contact Derm.* 1996, 35, 272–278.
- [20] Karlberg, A. T., Gäfvert, E., Lidén, C., *J. Am. Acad. Dermatol.* 1995, 33, 427–432.
- [21] Holmbom, B., Avela, E., Pekkala, S., *J. Am. Oil Chem. Soc.* 1974, 51, 397–400.
- [22] Smith, P. A., Gardner, D. R., Drown, D. B., Downs, G., Jederberg, W. W., Still, K., *Am. Ind. Hyg. Assoc. J.* 1997, 58, 868–875.
- [23] Jones, K., Garfitt, S. J., Calverley, A., Channa, K., Cocker, J., *Occup. Med.* 2001, 51, 507–509.
- [24] Ehrin, E., Karlberg, A.-T., *Contact Derm.* 1990, 23, 359–366.
- [25] Sadhra, S., Gray, C. N., Foulds, I. S., *J. Chromatogr. B* 1997, 700, 101–110.
- [26] Lee, B. L., Ong, H. Y., Koh, D., Ong, C. N., *J. Chromatogr. A* 1994, 685, 263–269.
- [27] Lee, B. L., Koh, D., Ong, H. Y., Ong, C. N., *J. Chromatogr. A* 1997, 763, 221–226.
- [28] Karlberg, A.-T., Lidén, C., *Contact Derm.* 1988, 18, 158–165.
- [29] Eriksson, K., Hagström, K., Axelsson, S., Nylander-French, L., *J. Environ. Monit.* 2008, 10, 345–352.
- [30] Fleck, E. E., Palkin, S., *J. Am. Chem. Soc.* 1937, 59, 1593–1595.
- [31] Ayer, W. A., Migaj, B. S., *Can. J. Bot.* 1989, 67, 1426–1428.
- [32] Karlberg, A.-T., Boman, A., Holmbom, B., Lidén, C., *Derm. Beruf Umwelt* 1986, 34, 31–36.
- [33] Lyon, C. C., Tucker, S., Gäfvert, E., Karlberg, A.-T., Beck, M. H., *Contact Derm.* 1999, 41, 102–103.