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Gas-Chromatographic Analysis of Oxygenated Terpenes in Toothpastes and Mouthwashes Using Solid Phase Extraction

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Introduction

Analysis of oxygenated terpenes in essential oils and natural products is a well documented problem, usually solved by means of gas-liquid chromatography (GC) (1–3) or high-performance liquid chromatography (HPLC) (4, 5). In the case of cosmetic formulations like toothpastes or mouthwashes, the quantification of terpenoids is a much more difficult task, owing to the complexity of these matrices. In addition to the abrasive material present in toothpastes, both formulations might contain water, humectants such as propylene glycol, glycerol, sorbitol or other polyols, ethanol, surfactants and foaming agents like sodium lauryl sulfate or sulfonated monoglycerides, carboxymethyl-cellulose or other thickening agents, natural or artificial sweeteners, preservatives, bacteriostatics and flavoring agents (6).

The main difficulty lies therefore in the quantitative isolation of the terpenoids from the matrix. This problem has been approached by few workers, who used either steam distillation with an organic solvent to take up the volatile compounds (7, 8) or liquid-liquid extraction with various solvents (9–11). Both techniques use large volumes of organic solvents and are very time-consuming, preventing their use on a routine basis. In addition, the former method hardly allows a satisfactory recovery of the volatile compounds during the distillation process, whereas the latter one yields an extract containing many disturbing coextractives.

To overcome these problems, we turned our attention to the solid-phase extraction technique, which is ideally suited to selectively extract compounds with a given functionality from complex matrices (12, 13). The principle of the proposed method is based on the separation of the terpenoid derivatives from cosmetic formulations using octadecyl-bonded (C_{18}) silica cartridges and ethanol as eluent, followed by quantitation using gas chromatography on Carbowax 20M packed column and flame-ionization detection (FID).

Experimental

Chemicals and samples

d-Camphor (purum), l-thymol (purum), eucalyptol (purum), menthol (puriss.) and cyclohexanol (puriss.) were purchased from Fluka AG (Buchs, Switzerland). Ethanol (94%, Ph. H. VI) was provided by Deresa SA (Lausanne, Switzerland), and water was distilled in an all-quartz apparatus.

Toothpastes, mouthwashes and mentholated candies were obtained from the local retail market.

Apparatus

Solid phase extraction was performed on 3 ml "Supelclean LC-18" cartridges (Supelco, no 5-7012) using a SPE vacuum manifold (Supelco).

Gas chromatography was performed on a Hewlett-Packard (Avondale, Pa, USA) model 5710A gas chromatograph equipped with a flame-ionization detector and coupled to a Hewlett-Packard model 3390A integrator. Injector and detector temperatures were 150 °C and 250 °C, respectively. The glass column (1.65 m × 2 mm ID) was packed with 5% Carbowax 20M on Chromosorb W-HP (80–100 mesh). Nitrogen was used as carrier gas at a flow rate of 37.5 ml/min ($T = 25$ °C). Temperature was programmed from 60 °C to 170 °C at a rate of 16 °C/min.

Sample preparation

Toothpastes and candies

2 g of homogenized sample are accurately weighted in a 25 ml centrifuge tube, to which are added 5 ml water/ethanol 1:1 by volume. The suspension is mixed for 2 minutes and centrifuged, and the supernatant is transferred into a 25 ml volumetric flask. The extraction procedure is repeated twice, and 1 ml of a 5% (w/v) solution of cyclohexanol (internal standard) in water/ethanol is added to the volumetric flask, which is completed to mark with water/ethanol.

Mouthwashes

500 μ l of sample are mixed with 500 μ l of cyclohexanol 0.08% (w/v) in water/ethanol.

Sample clean-up

1. To condition the "Supelclean LC-18" cartridge, add 1 ml ethanol, and turn vacuum on. Immediately follow the ethanol addition with 1 ml of water, and when it has run from the cartridge turn vacuum off.

2. Add 1 ml of the solution to analyze and aspirate through the cartridge.
3. Rinse cartridge with 3 ml of water and turn vacuum off.
4. Position an appropriately labelled 5 ml volumetric flask in the rack within the vacuum manifold.
5. Add 4 ml of ethanol in two portions and release the vacuum after complete collection of eluant.
6. Adjust the volume of the volumetric flask to 5. ml.

Chromatographic analysis

1 μ l of the final extract is injected in the chromatograph. A standard solution containing 0.04% (w/v) of the terpenoids and cyclohexanol (internal standard) is prepared in water/ethanol, and 1 μ l is injected in the chromatograph. The concentration of terpenoids in the sample is then given by the following formula:

$$C_{AE} = C_{AS} \cdot F \cdot (H_{AE} \cdot H_{SS}) / (H_{SE} \cdot H_{AS})$$

H_{SS} = Height of cyclohexanol peak in standard solution.

H_{AS} = Height of product A in standard solution.

H_{SE} = Height of cyclohexanol peak in sample solution.

H_{AE} = Height of product A in sample solution.

C_{AE} = Concentration of A in sample.

C_{AS} = Concentration of A in standard solution.

F = Dilution factor.

Results and discussion

Due to the pharmacological properties of oxygenated terpenes, their use in cosmetic formulations is restricted by Swiss regulation (14, 15). The upper limit allowed for these compounds depends on the type of cosmetic : group A includes formulations in contact with mucous membranes (e. g. toothpaste or mouth-wash), group B formulations which remain on the skin (e. g. make-up, skin-cream or ointment), and group C formulations which are removed after a short application time (e. g. shampoo or hair-dye). The maximum terpenoid concentrations allowed for these three groups are given in table 1.

Table 1. Maximum limits for terpenoids in cosmetic formulations (values are given in % by weight)

Terpenoid	Group A	Group B	Group C
Camphor	1.0	3.0	4.0
Eucalyptol	1.0	1.0	
Menthol	1.0	1.0	
Thymol	0.1	0.3	

In practice, oxygenated terpenes are found in toothpastes, mouthwashes and ointments used as counterirritants and muscle relaxants. To get rid of the analytical problems mentioned in the introduction, we investigated a clean-up procedure based on solid phase extraction, using bonded-phase silica cartridges. The principle of this so-called sorbent extraction is based on the greater affinity of the analyte for the solid phase than for the solvent in which it is dissolved, while other compounds are not retained by the derivatized silica. A careful choice of the sorbent can therefore provide a fine-tuning of the interaction mechanism between analyte and bonded-phase, leading to very specific isolations.

To test the extraction process, we used a 0.1% (weight by volume) solution of camphor, menthol, eucalyptol and thymol in water/ethanol 1:1 (by volume), to which cyclohexanol at a final concentration of 0.1% was added as internal standard. All these compounds bear the same functionalities, namely a polar group (ether, carbonyl or alcohol functions) and a hydrophobic part represented by the terpene skeleton. We first tried to exploit a polar interaction by using a diol cartridge, but too many compounds were retained in addition to the analytes, i. e. the selectivity was too low for this type of sorbent. We then tried two hydrophobic cartridges, with octyl (C_8) and octadecyl (C_{18}) phases. The analyte recovery was measured by GC-FID on a Carbowax 20M phase (see experimental part for GC conditions). For C_8 sorbent, washings contained significant amounts of analytes, whereas all compounds were quantitatively retained on C_{18} phase.

To check the effectiveness of the proposed method, we applied it to a cinnamon mouthwash fortified with 0.25% of all compounds under study. The absence of analysed terpenoids in that mouthwash was previously ascertained by gas chromatography/mass-spectrometry. Recoveries obtained were better than 97%. The same procedure was then applied four times to a mentholated mouthwash fortified with 0.05% (assays 1 and 2) and 0.25% (assays 3 and 4), and recoveries were calculated by comparison with standards of known concentrations. The results of recovery tests are given in table 2.

Table 2. Recoveries for terpenoid compounds in fortified mouthwash (values are given in %)

Compound	Assay 1	Assay 2	Assay 3	Assay 4	Mean
Eucalyptol	90.9	97.4	102.1	109.4	99.9
Camphor	99.1	96.0	99.6	95.9	97.6
Menthol	100.2	99.4	100.9	104.9	101.4
Thymol	94.9	96.8	96.9	101.4	97.5

Retention indices of terpenoids are shown in table 3. Limits of detection were in the order of 1 mg/l, corresponding to 1 ng for a 1 μ l injection. They were obtained by running an ethanolic solution of the compounds under study through

the extraction process, and were calculated as a signal 3 times greater than background standard deviation (16). For a calculation of detection limits in the studied matrices, these figures have of course to be multiplied by the dilution factors arising from the sample preparation.

Table 3. Retention characteristics of terpenoid compounds

	Eucalyptol	Camphor	Menthol	Thymol
Retention time/min	2.31	4.82	5.70	10.71
Relative Rt ¹	0.60	1.25	1.48	2.78

¹ Retention time of cyclohexanol = 1.00.

The method was then applied to four mouthwashes, four toothpastes and one mentholated candy. Results of these analyses are shown in table 4, whereas figure 1 shows a chromatogram of a standard solution and that of an extract of brand A mouthwash.

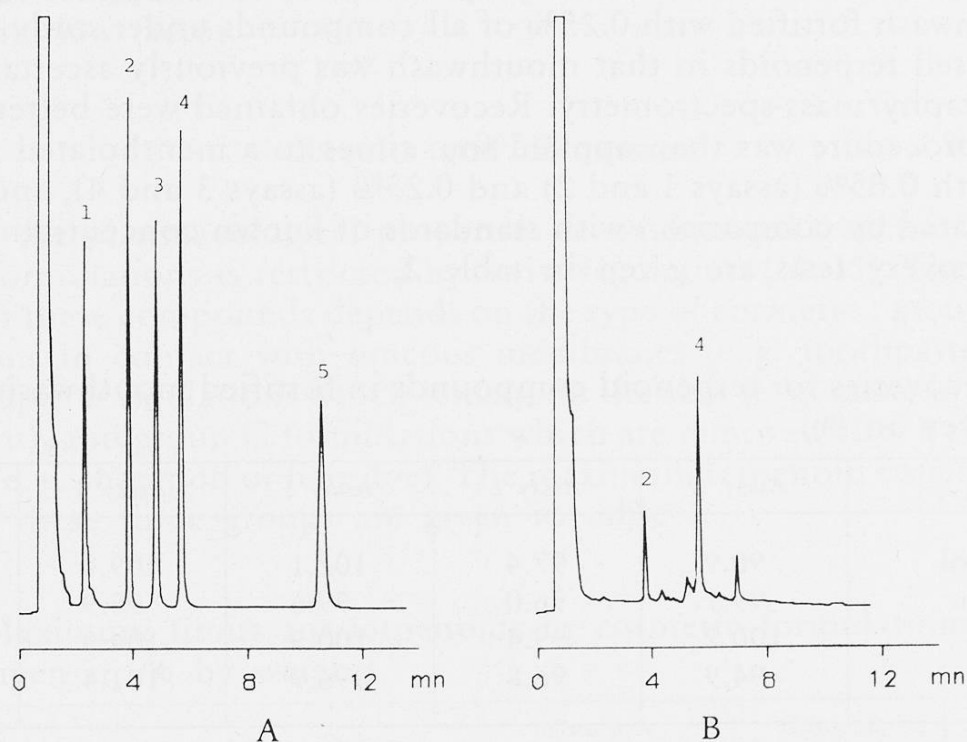


Fig. 1. A = Chromatogram of a mixed standard solution. B = Chromatogram of a mouthwash extract. 1 = Eucalyptol, 2 = Cyclohexanol, 3 = Camphor, 4 = Menthol and 5 = Thymol. Chromatographic conditions: see experimental part.

Table 4. Results of terpenoid analyses in various samples¹ (values are given in %)

Sample	Menthol	Sample	Menthol
Mouthwash A	0.14	Toothpaste A	0.24
Mouthwash B	0.95	Toothpaste B	0.22
Mouthwash C	2.76	Toothpaste C	0.35
Mouthwash D	< 0.01 ²	Toothpaste D	0.33
Mentholated candy	0.23		

¹ In all samples analyzed, only menthol was found.

² Cinnamon-flavored mouthwash.

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Summary

The present work proposes a rapid method for the simultaneous analysis of thymol, menthol, eucalyptol and camphor in cosmetic formulations. Samples are extracted in water/ethanol 1:1 (v/v), cleaned-up on a C₁₈ solid-phase extraction cartridge, and analysed by GC on a Carbowax 20M packed column. Recoveries are better than 97%, and the limit of detection lies in the order of 1 mg/l for the extract.

Zusammenfassung

Die vorliegende Arbeit schlägt eine schnelle Methode für die gleichzeitige Bestimmung von Menthol, Thymol, Eukalyptol und Kampfer vor. Die Extraktion erfolgt in einem Gemisch Ethanol-Wasser 1:1 (vol/vol), die Reinigung auf einer Extraktionshülse mit C₁₈ als feste Phase und die Bestimmung auf einer mit Carbowax 20M gefüllten GC-Säule. Die Wiederfindungsrate beträgt mehr als 97%, und die Nachweisgrenze liegt, auf den Extrakt bezogen, bei 1 mg/l.

Résumé

Le présent travail propose une méthode rapide de dosage simultané de menthol, thymol, eucalyptol et camphre dans les cosmétiques. Les échantillons sont extraits en milieu éthanol/eau 1:1 (v/v), purifiés sur cartouche d'extraction à phase solide C₁₈, et dosés par GC sur colonne remplie de Carbowax 20M. Les taux de récupération sont supérieurs à 97%, et la limite de détection est de l'ordre de 1 mg/l, exprimée sur l'extrait.

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