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Analysis of synthetic mixtures of waxes by supercritical fluid chromatography with packed columns using evaporative light-scattering detection

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ABSTRACT

The analysis of waxes, the basis of numerous cosmetic products, is frequently carried out by gas chromatography. Unfortunately, the chromatographic "fingerprints" are very complex and sometimes abnormal compositions, a sign of "doping" of waxes, remain undetected. The object of this study by supercritical fluid chromatography with packed columns was to select chromatographic conditions that characterize the majority of the different wax families and not a separation according to the hydrocarbon chain for a given family.

INTRODUCTION

Natural waxes, which are very complex mixtures mainly consisting of hydrocarbons, esters, triglycerides, alcohols and acids with long-chain alkyl groups in the range C_{20} to $>C_{50}$ (straight, branched, saturated or unsaturated), are commonly used in the cosmetics industry because of their many advantageous properties. The compositions of different waxes such as candellila or carnauba have long been known [1,2]. Their analysis by gas chromatography (GC), is often carried out on capillary [3] or packed columns [1,4], but the chromatograms remain exceedingly complex. The systematic identification of each component is lengthy and sometimes impossible. This technique often requires the derivatization of the more polar components, so that alcohols are frequently converted into their acetate derivatives after treatment with acetic anhydride and acids into their methyl esters after treatment with diazomethane [1–6]. Moreover, insufficient volatility of certain components such as triglycerides requires working at high temperatures, adding the risk of degradating certain thermally labile compounds.

Different synthetic mixtures of waxes have been analysed by supercritical fluid chromatography (SFC) using packed columns, the first objective being to obtain the chromatographic "fingerprint" whereby each family of solutes is represented by a single signal, the total representing the bulk characteristics. For this reason, the system had to be capable of resolving the functional groups and not the methylene groups.

Constituent waxes are either slightly UV absorbing or do not absorb at all. It is also necessary to use universal detection in order to avoid the preliminary derivatization steps. Evaporative light-scattering detection (ELSD) seemed to be suitable for this type of study because of its compatibility with SFC [7–9]. Also, contrary to the frequently used flame ionization detection (FID), it is possible to add one or several polar modifiers to the supercritical carbon dioxide to elute all wax compounds without any residue problems.

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This paper discusses the influence of different parameters such as stationary phases, temperature and chromatographic column pressure on the elution of synthetic wax mixtures.

EXPERIMENTAL

Two sets of apparatus were used, as described below.

First apparatus

The SFC apparatus was set up as follows. Carbon dioxide was pumped with a Waters (Milford, MA, USA) Model M 501 pump. An ethanol cooling bath was used to cool the pump head in order to increase the pump-down efficiency. A polar modifier was added using a Jasco (Tokyo, Japan) Model 2510 pump. The two solvents were mixed at a controlled temperature (Jasco 860-CO oven) in a dynamic mixing chamber (Knauer, Berlin, Germany). A Rheodyne (Touzart et Matignon, France) Model 7125 injection valve, with a 20-μl loop, and the column were placed in the Jasco oven. A Touzart et Matignon Model 45 evaporative light-scattering detector with the interface adjusted to SFC was used.

Second apparatus

Carbon dioxide was pumped with a Gilson (Villiers-le-Bel, France) Model 305 piston pump. An ethanol cooling bath was used to cool the pump head. The polar modifier was added using a Gilson Model 306 piston pump. The two solvents were mixed at ambient temperature in a Gilson Model 811 mixer. A Gilson Model 821 pressure regulator, controlled by the Model 305 pump, was placed between the column and the restrictor. A Rheodyne Model 7125 injection valve with a 20-μl loop and the column were placed on the outside of the Knauer oven. A Touzart et Matignon Model 45 evaporative light-scattering detector was used.

Chromatographic column

Various stationary phases and columns were used: 7-μm LiChrosorb diol (250 mm × 7 mm I.D.); 5-μm LiChrosorb diol (150 mm × 4.6 mm I.D.); 5-μm LiChrospher 100 diol (125 mm × 4 mm I.D.) (Merck, Darmstadt, Germany); and 5-μm Zorbax diol (250 mm × 9.4 mm I.D.) (DuPont, Wilmington, DE, USA).

Chemicals and reagents

Carbon dioxide was of B 50 grade purchased from Air Liquide (Paris, France). 2-Propanol (SDS, Peypin, France) and acetone (Fisons, Loughborough, UK) were of HPLC grade and other polar modifiers were of analytical-reagent grade. Triglyceride (tripalmitine and tristearine in mixture) and stearic acid solutes were purchased from Fluka (Buchs, Switzerland), stearic alcohol from Baker (Deventer, Netherlands) and cetyl myristate from Fluka.

RESULTS AND DISCUSSION

The initial analysis and characterization of waxes were limited to determining the main components according to each chemical group rather than its alkyl chain length. Polar stationary phases were chosen even when the amount of the apolar solutes remained predominant in each family because selectivity, according to the apolar function, is poor on these phases, and therefore advantageous in the analysis of the more polar groups. Using bare silica columns involved long equilibrium times, so diol-bonded silicas (Zorbax, LiChrosorb, LiChrospher) were employed.

The first studies were carried out using carbon dioxide at a constant flow-rate of 3 ml/min (see Experimental, first apparatus). Working pressure was maintained by a specially made outlet restrictor of fused silica placed between the chromatographic column and the detector which nebulizes the fluid at its end. This system, recently employed in several studies, was preferred to Guthrie tubing [10], which is brittle, tends to clog and often requires replacement. The addition of an organic modifier to the carbon dioxide (acetone, 2-propanol, formic acid) permitted the elution of less polar compounds such as esters, triglycerides and the more polar compounds such as alcohols and acids. In spite of this and because of weak retentions of esters and triglycerides on an analytical-size column, wider diameter columns were necessary for better resolutions. The greater phase volume of these columns made it possible to vary the amount (x%) of organic modifier within a close range, thus avoiding large retentions of the more polar compounds where x was too low and too rapid elution where x was very high. Consequently, small variations in the organic modifier content help to avoid major variations in pressure and eluent density.

As shown in Fig. 1a and b, on the Zorbax diol column, an increase in 2-propanol content of the mobile phase was necessary to elute the esters, triglycerides and alcohols. On the other hand, too much 2-propanol, needed to elute the organic acid in isocratic elution, brought the risk of mixing the other three components with too rapid elution. Moreover, the peak shape of this acid was asymmetric, indicating strong adsorption. Berger and Deye [11] have recently shown that polar organic acids, such as certain basic solutes such as amines, were difficult to elute by packed-column SFC. By adding a small amount of a strong acid such as formic acid to the mobile phase, the interactions of the residual silanol groups of the stationary phase with the analytes were decreased, resulting in their elution with a good peak shape without eluting the other solutes too rapidly (Fig. 1c).

Selectivity between the four probe solutes had to be improved so that each chromatographic peak could represent a family having any hydrocarbon chain length. Therefore, acetone was tried as modifier instead of 2-propanol because of its lowest hydrogen bond solubility parameter ($\delta_h = 3.4$ [12]) than that of 2-propanol (8.0) and its higher dipole–dipole solubility parameter ($\delta_p = 5.1$) than that of 2-propanol (3.0). From a comparison of Figs. 2a and 1b, it can be concluded that acetone had a lower eluent strength than 2-propanol and the acid solute was not eluted. In the same way, as shown in Fig. 1c, this modifier necessitated adding a small amount of formic acid (Fig. 2b). In this instance, the same effect was obtained as with 2-propanol and the acid was eluted without changing the selectivity of the other three solutes.

In addition, if we compare the selectivities α (Table I) relating to the ester from Figs. 1c and 2b, the following observations can be made: acetone as a polar modifier eluted the carbonyl derivatives such

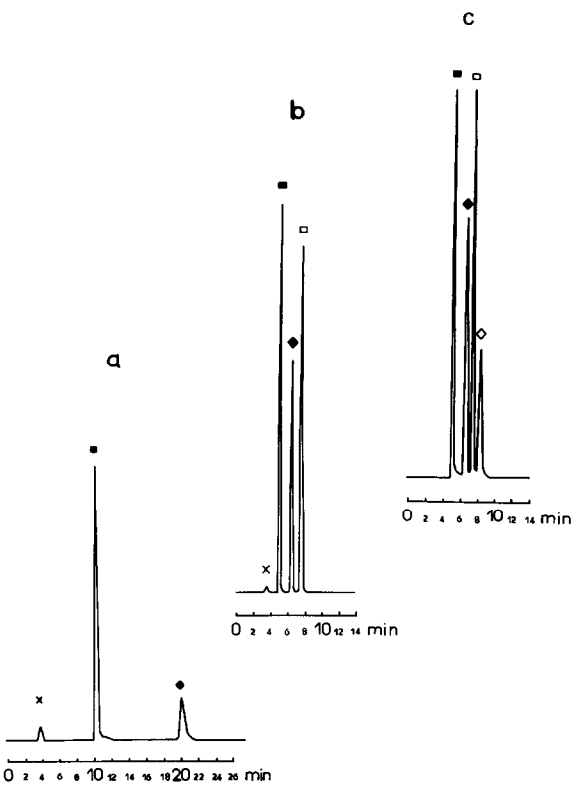

Fig. 1. Separation of (■) cetyl myristate, (◆) triglycerides (cetyl and octadecyl chain in mixture), (□) stearylic alcohol, (◇) stearic acid and (×) chloroform (injection solvent). Chromatographic conditions: column, Zorbax diol (250 mm × 9.4 mm I.D.); mobile phase, (a) carbon dioxide–2-propanol (96.8:3.2, w/w), (b) carbon dioxide–2-propanol (91:9, w/w) and (c) carbon dioxide–2-propanol–formic acid (90.9:8.8:0.3, w/w/w); flow-rate, 3.3 ml min^{-1}; pressure, 130 bar; detection, ELSD.

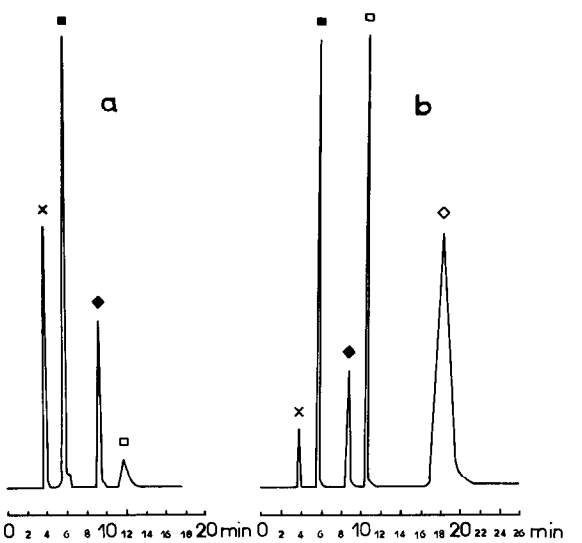

Fig. 2. Separation of (■) cetyl myristate, (◆) triglycerides (cetyl and octadecyl chain in mixture), (□) stearylic alcohol, (◇) stearic acid and (×) chloroform (injection solvent). Chromatographic conditions: column, Zorbax diol (250 mm × 9.4 mm I.D.); mobile phase, (a) carbon dioxide–acetone (91:9, w/w), (b) carbon dioxide–acetone–formic acid (90.9:8.8:0.3, w/w/w); flow-rate, 3.3 ml min^{-1}; pressure, 130 bar; detection, ELSD.

TABLE I

SELECTIVITY RELATING TO THE ESTER

For chromatographic conditions, see Figs. 1c and 2b.

Organic modifier	Content (%)	α		
		Triglycerides	Alcohol	Acid
2-Propanol	8.80	2.13	2.72	3.42
Acetone	8.80	2.66	3.60	7.70

as 2-propanol (see Table I, $\alpha_{triglycerides/ester}$ have similar values) and 2-propanol eluted the hydroxyl derivatives (alcohol and acid) more easily than acetone (see Table I). These remarks have to be considered in relation to the aforementioned components of the solubility parameters.

To avoid adding formic acid in the mobile phase, a few other diol-bonded silicas phases were tried, especially LiChrosorb in a wide-diameter column. As shown in Fig. 3, the four components were eluted with the carbon dioxide–2-propanol binary mixture and the retention decreased with an increase in 2-propanol content. With a 4.68% 2-propanol content an excellent separation was efficiently obtained (Fig. 4) in about 11 min with a reduced plate height (h/d_p) of 5 (where h and d_p are the plate height and particle size, respectively). The asymmetry factor, A_s, measuring the tailing band at 10% of the acid peak height decreases from 5 to 3 when the 2-propanol content varies from 3.17 to 4.68%.

In certain waxes where the more polar compounds such as triglycerides, alcohols and acids are in the minority, it is difficult to determine their concentrations when the less apolar components such as hydrocarbons and esters are predominant. For this reason, it is advisable to isolate the less polar derivatives in order to obtain a richer mixture. This extraction must be carried out with carbon dioxide in order to circumvent contamination by the solvents.

The following two modifications were made to avoid too long retention times on these diol-bonded silicas using carbon dioxide: wider diameter columns (250 mm × 9.4 or 7 mm I.D.) were replaced with analytical-size columns (150 mm × 4.6 mm I.D. or 125 mm × 4 mm I.D.); and a programmable Gilson pressure regulator (see Experimental, second apparatus) ensuring mobile phase flow and a constant pressure was placed at the column outlet just

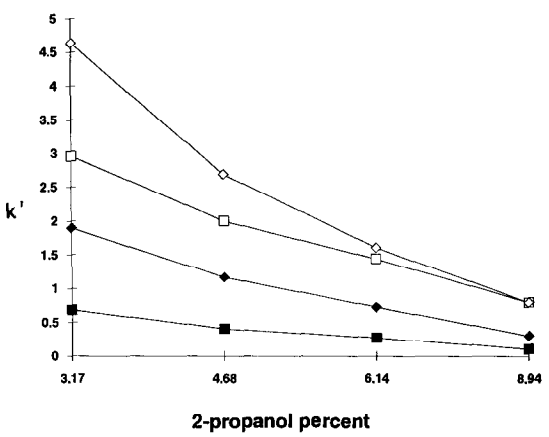

Fig. 3. Graph of k' *versus* percentage of 2-propanol. ■ = Cetyl myristate; ◆ = triglycerides (cetyl and octadecyl chain in mixture); □ = stearylic alcohol; ◇ = stearic acid. Chromatographic conditions: column, LiChrosorb diol (250 mm × 7 mm I.D.); mobile phase, carbon dioxide–2-propanol; flow-rate, 3.3 ml min^{-1}; pressure, 130 bar; detection, ELSD.

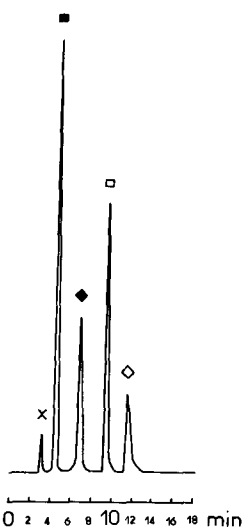

Fig. 4. Separation of (■) cetyl myristate, (◆) triglycerides (cetyl and octadecyl chain in mixture), (□) stearylic alcohol, (◇) stearic acid and (×) chloroform. Chromatographic conditions: column, LiChrosorb diol (250 mm × 7 mm I.D.); mobile phase, carbon dioxide–2-propanol (95.3:4.7, w/w); flow-rate, 3 ml min^{-1}; pressure, 140 bar; detection, ELSD.

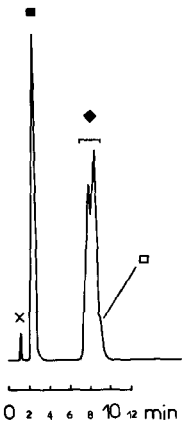

Fig. 5. Separation of (■) cetyl myristate, (◆) triglycerides (cetyl and octadecyl chain in mixture), (□) stearylic alcohol and (×) chloroform. Chromatographic conditions: column. LiChrosorb diol (150 mm × 4.6 mm I.D.); mobile phase, pressure gradient from 150 bar carbon dioxide (flow-rate 3 ml min^{-1}) to 250 bar carbon dioxide (flow-rate 4 ml min^{-1}) in 25 min; detection, ELSD.

ahead of the restrictor; because of this regulator, the carbon dioxide density and therefore its polarity could vary within wide ranges.

After a preliminary study on the satisfactory LiChrosorb diol support, we observed (Fig. 5) poor selectivities of the triglycerides and alcohols, and the acids were not eluted. However, the beginning of a triglyceride separation according to its alkyl chain length was noted. LiChrospher diol, a similar but spherical support, was chosen with seemingly more success because all four families of the components could be eluted. Fig. 6, showing the effect of the pressure on the capacity factor (k') variation, indicates that, at low pressure (166 bar), the ester was easily eluted whereas the triglycerides and acids were not fully separated. Their retentions were high and their asymmetry factor, A_s, too great. Note here the retention inversion in Fig. 4. At high pressure (290 bar), retentions decreased and no alcohols or triglycerides were separated. By programming the pressure regulator (Fig. 7), it was possible to elute the ester at low pressure and the other three polar components under higher pressures with good resolutions.

Temperature was the final parameter to be studied. Similarly to liquids, a solute at any given temperature is more soluble in a supercritical fluid than in a gas. What we observed here is different from the aforementioned effects of temperature on retentions. Huston and Berhard [13] explained that

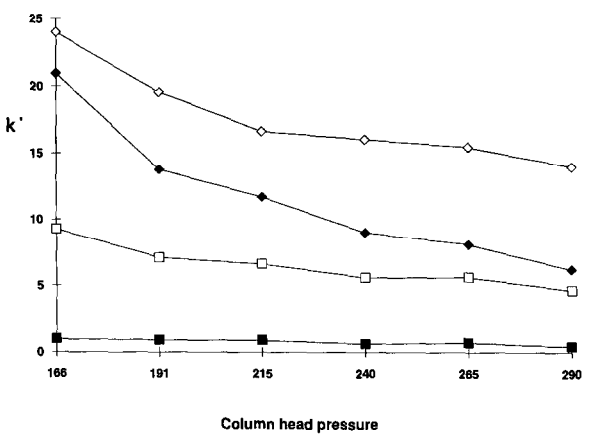

Fig. 6. Graph of k' *versus* pressure (bar). ■ = Myristyl myristate; ◆ = triglycerides (cetyl and octadecyl chain in mixture); □ = stearylic alcohol; ◇ = stearic acid. Chromatographic conditions: column, LiChrospher diol (125 mm × 4 mm I.D.); mobile phase, carbon dioxide; flow-rate, 3 ml min^{-1}; detection, ELSD.

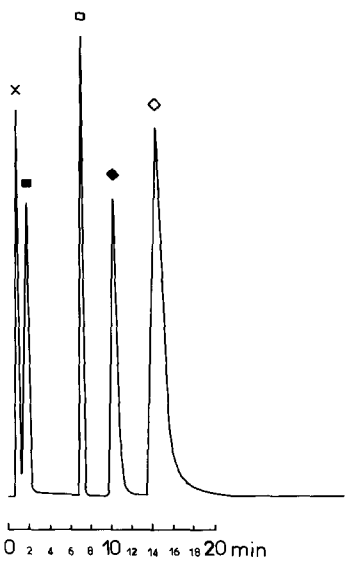

Fig. 7. Separation of (■) myristyl myristate, (□) stearylic alcohol, (◆) triglycerides (cetyl and octadecyl chain in mixture), (◇) stearic acid and (×) chloroform. Chromatographic conditions: column, LiChrospher diol (125 mm × 4 mm I.D.); mobile phase, pressure gradient from 140 to 275 bar in 10 min; flow-rate, 3 ml min^{-1}; detection, ELSD.

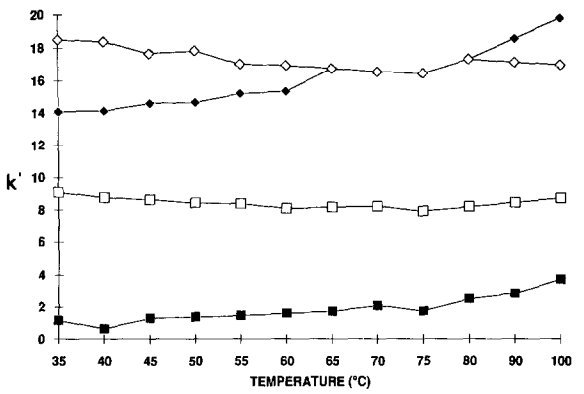

Fig. 8. Graph of k' *versus* temperature. ■ = Cetyl myristate; □ = stearylic alcohol; ◆ = triglycerides (cetyl and octadecyl chain in mixture); ◇ = stearic acid. Chromatographic conditions: column, LiChrospher diol (125 mm × 4 mm I.D.); mobile phase, carbon dioxide; flow-rate, 3 ml min^{-1}; column head pressure, 166 bar; detection, ELSD.

such a curve shows a maximum. When the temperature increases, the mobile phase density decreases. In the first part of the curve, increased retention was caused by a decrease in the solubility of the solute in the mobile phase. In the second part of the curve, beyond the retention maximum temperature, while solubility continued to decrease, a higher temperature caused an increase in volatility and so the solutes were not retained as much. A smaller variation in selectivity for the triglycerides–stearic acid solute pair could be observed from 65°C upwards (Fig. 8). None of the previous temperature effects were noted, with the only difference being that these studies were carried out with capillary columns. In conclusion, a slight evolution of the k' values indicates that temperature has less influence than pressure. The best selectivities may be obtained by working at temperatures lower than 60°C or higher than 90°C. As high temperatures are not desirable for long-term column stability and because of thermal degradation of the solutes, all subsequent experiments were performed at 45°C.

CONCLUSIONS

From these preliminary studies on polar stationary phases, chromatographic systems have been developed that make it possible to identify the main component types encountered in waxes.

The two systems employed were as follows: without pressure programming, 7-μm LiChrosorb diol column (250 mm × 7 mm I.D.), mobile phase carbon dioxide–2-propanol (96.83:3.17, w/w), flow-rate 3.1 ml min^{-1} and pressure 124 bar; and with pressure programming, 5-μm LiChrospher 100 diol column (125 mm × 4 mm I.D.), mobile phase carbon dioxide pressure initially 165 bar and flow-rate 3 ml min^{-1} to 10 min pressure 290 bar and flow-rate 3 ml min^{-1}. The latter system appears to be the most advantageous owing to a shortened column equilibrium time and the fact that FID can be used.

The application of the above method to the analysis of commercial waxes is in progress but it will probably require supercritical extraction of the major components of waxes such as hydrocarbons (not treated here) and esters in order to achieve the preconcentration of the minor species, namely triglycerides, alcohols and acids.

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