

# Capillary Supercritical Fluid Chromatography of Cosmetic Ingredients and Formulations

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**Abstract:** Capillary supercritical fluid chromatography (SFC) is an extremely versatile technique for the characterization of natural products that are used in the cosmetics industry. In this study, the utility of capillary SFC for separating components contained in natural lanolin, cosmetic grade lanolin, and lanolin derivatives and for profiling the components in cosmetic formulations, such as lipsticks and lip balms, is demonstrated. Capillary SFC is also shown to be facile for separating reaction products from starting materials for substrates frequently used in cosmetic formulating. Optimal stationary phases for the separation of nonpolar and lipophilic components found in cosmetic products have been determined. SFC profiles generated on SB-octyl columns using linear density programs have been particularly useful for cosmetic deformation. Retention data on complex natural mixtures correlate with the overall polarity of the solutes and traditional SFC retention patterns based on the molecular weight of the solutes. © 1998 John Wiley & Sons, Inc. *J Micro Sep* 10: 33–39, 1998

**Key words:** *capillary; chromatography; cosmetics; lanolin; supercritical fluid*

## INTRODUCTION

Supercritical fluid chromatography (SFC) in its diverse modes, i.e., capillary [1], packed capillary [2], packed column [3], low resolution packed column [4], and preparative, have contributed significantly to the enhancement of analytical separation science [5] in recent years. Besides SFC's intrinsic separation capability, it has been demonstrated that SFC can contribute to allied fields such as supercritical fluid extraction [6] as well as our understanding of the physicochemical properties of supercritical fluids, as demonstrated by Giddings et al. [7] over 30 years ago. However, it was not until the development of practical capillary SFC by Novotny and Lee [8] and Lee et al. [9] that SFC could be integrated into routine use in the analytical laboratory.

Complex natural and commercial product sample matrices represent a challenge to the high resolving power of the currently manufactured capillary SFC columns, and as noted by Giddings [10],

multidimensional techniques do not always serve as a panacea for the resolution of mixtures containing components with similar physical and chemical properties. Nonetheless, multidimensional methods have proven applicable in many cases, as recently documented by Tong [11], Mondello [12], and Greibrokk [13]. In this regard, many cosmetic ingredients derived from natural product sources [14] or commercially formulated cosmetic products represent a significant challenge to many high-resolution chromatographic methods, either on a singular or multidimensional basis.

Capillary SFC has been applied for cosmetic ingredient analysis as documented by the studies of Broadbent et al. [15], Tong et al. [16], and Nicolae and Bryant [17]. We have also demonstrated the applicability of capillary SFC in our laboratories for the characterization of jojoba oil and derivatives [18] as well as to study the supercritical fluid extraction (SFE) of crude lanolin from wool grease [19]. As we have noted before [20, 21], SFC is particularly well suited for the rapid characterization of raw materials that are incorporated into commercially formulated cosmetic products as well as an adjunct method for characterizing extracts [22] and/or reaction prod-

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ucts [23] derived from supercritical fluid media. In this research we have extended our studies to characterize specific derivatives derived from natural products which have utility in cosmetic formulations as well as to extend the use of capillary SFC to the deformation of commercial cosmetic products containing lipophilic ingredients.

In addition, besides the value to applied technology, such studies permit the analyst to study the interaction between complex mixtures of chemically similar or dissimilar solutes and common capillary SFC stationary phases, thereby choosing phases which are optimal for a given sample or having widespread applicability for a variety of sample types encountered in the cosmetic industry. We believe this is a somewhat different approach that makes more practical sense than studying the retention of model solutes on different column packings in SFC [24, 25].

#### EXPERIMENTAL

Chromatographic experiments were performed on a Lee Scientific model 501 SFC unit (Dionex Corporation, Sunnyvale, CA) equipped with a flame ionization detector (FID) controlled by an IBM PC-AT computer. The output signal from the FID was recorded on an Omniscrite recorder (Houston Instruments), while hardcopy of the CRT control screen was printed on a Panasonic KX P1091 printer. The column mobile phase was carbon dioxide (CO<sub>2</sub>) obtained from Scott Speciality Gases (Plumsteadville, PA), and it was further purified by passage over an in-line cleanup trap placed before the syringe pump of the model 501. The trap was filled with basic alumina (Brockmann I, Aldrich Chemical, Milwaukee, WI) that had been activated at 450°C overnight.

All injections were performed with the aid of a Valco valve, cooled to approximately -10°C, having an internal injection cavity of 200 nL. Injection times of 12 s were used to transfer the contents of the injector onto the column proper. The FID was maintained at 350°C or 375°C. Frit restrictors (Dionex Corporation, Sunnyvale, CA) were used to maintain back pressure on the capillary column. For most of the reported separations, initial mobile phase velocities were 2 or 4 cm/s. The injected samples were dissolved in *n*-hexane (1-10 wt%), augmented by the addition of 1-2 drops of either methylene chloride or acetone, to remove any evidence of turbidity in the injected solution.

Table I lists the various capillary columns that were evaluated during this study. As one can see from Table I, these were typically 10-15 m in length, 50 μm internal diameter, and had stationary phase

**Table I.** Capillary SFC columns utilized in this study.

Stationary phase	Length (m)	Diameter (μm)	Film thickness (μm)
SB-Methyl	10	50	0.25
SB-Octyl	15	50	0.25
SB-Biphenyl 30	10	50	0.25
SB-Cyanopropyl 50	10	50	0.25
SB-Smectic	5	50	0.15

film thicknesses of 0.25 μm. As will be discussed in the Results and Discussion section, the most successful separations were usually obtained on the more nonpolar phases; the SB-methyl, SB-octyl, and SB-biphenyl 30 stationary phases. This is undoubtedly due to the high content of nonpolar, hydrophobic-type solutes contained in the samples we chose to chromatograph.

Table II describes the density programs used to affect the separation of the solutes contained in the various samples that were chromatographed. These programs were designed to afford the highest resolution possible, consistent with a reasonable analysis time; they tended to average approximately 90 min in length. It should be noted that density programs of a shorter time duration could be used after surveying the complexity of the natural product mixture or cosmetic formulation, but for the purposes of this study, the elongated programs proved more facile, particularly when comparing one commercial formulation against another. Peak identifications were assigned based on matching retention times with standard solutes and a prior knowledge as to the content of the mixture injected.

**Table II.** Density program utilized in performing separations.

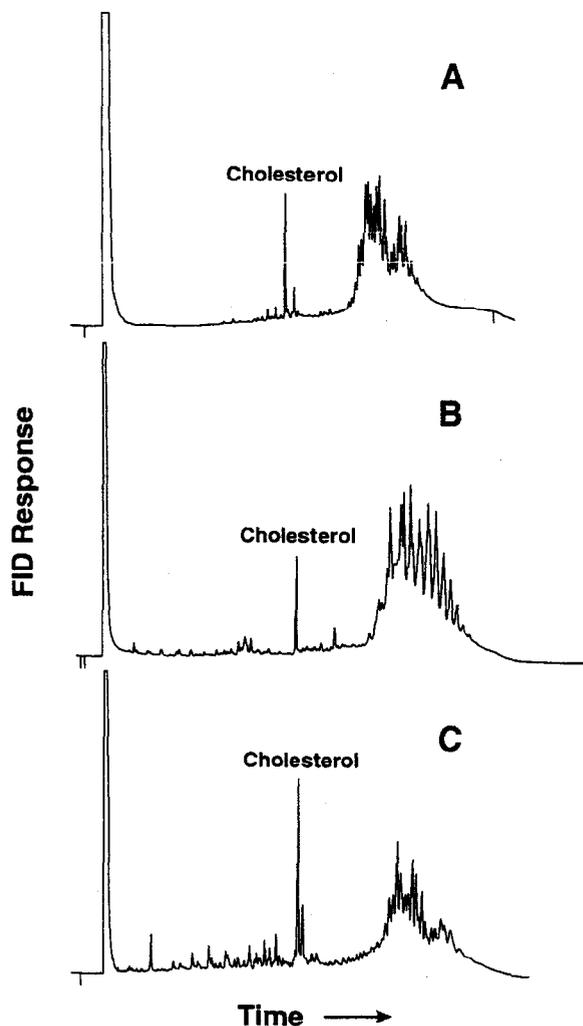
Program 1
Oven temperature 120°C
Initial density 0.28 g/mL
Initial hold time 15 min
Density ramp rate 0.006 g/mL/min
Final density 0.66 g/mL
Final hold time 15 min
Total run time 90.17 min
Program 2
Oven temperature 100°C
Initial density 0.15 g/mL
Initial hold time 15 min
Density ramp rate 0.01 g/mL/min
Final density 0.76 g/mL
Final hold time 10 min
Total run time 96.72 min

Commercial lanolin samples and their chemical derivatives were obtained from the Rita Corporation (Crystal Lake, IL) and Croda, Inc. (New York City, NY). Highly purified cosmetic grade fatty acids and alcohols were also provided gratis from Croda, Inc. Cosmetic and personal care products used in these studies were purchased from local drugstores and groceries.

## RESULTS AND DISCUSSION

**Lanolin and lanolin derivatives.** Lanolin is a "fat-like" secretion from the sebaceous glands of the sheep [26] which consists of a complex mixture of particularly high molecular weight (790–880 daltons) esters and polyesters. The molecular complexity of this waxlike substance has defied for many years the best attempts of chemists to unravel its structure. Lanolin and its chemical derivatives are widely employed in numerous cosmetic formulations and serve as a critical test of the resolving power afforded by neat capillary SFC using commercially available columns. As will be shown later, obtaining an "SFC signature" of commercial lanolin is critical for identification of its presence in commercial cosmetic formulations.

As can be seen from Figures 1(a)–(c), capillary SFC provides some resolution of components in this "natural" cosmetic ingredient. As shown in all of the chromatograms in Figure 1, lanolin contains cholesterol as a major peak followed by an unresolved cluster of predominately wax ester moieties. SFC also affords adequate resolution of a number of minor components which are present in small quantities in lanolin and that elute before the wax ester profile. In general, it was found that the SB-octyl and SB-methyl columns were superior to the polar bonded phases for resolving many of the components found in lanolin and its chemical derivatives. A comparison of the lanolin profiles obtained under identical programming conditions [program 1, Figures 1(a)–(c)] indicated that the elution profile remained unchanged; however, shifts in certain peaks were discernible. Certain components in the wax ester profile appear to elute in a more regular pattern on the SB-biphenyl-30 column, resulting in larger peaks. Application of asymptotic density programming to the lanolin samples resulted in only minor improvement to the wax ester profile. Based on this observation, the SB-octyl column was judged to be the most suitable column for further studies of lanolin derivatives, since it offered the best resolution possible of



**Figure 1.** Capillary SFC separation of cosmetic grade lanolin using different columns: (A) SB-methyl, (B) SB-Biphenyl-30, (C) SB-octyl.

the base profile of lanolin [Figures 1(a)–(c)], and components present in its derivatives, which are usually of lower molecular weight.

Acetylation of lanolin is accomplished via reaction of acetic anhydride with compounds having free hydroxyl groups. This converts the original lanolin to an esteric composition with a marked increase in oil solubility. Modification of the hydroxyl groups via acetylation also eliminates the emulsifying properties of lanolin. Capillary SFC shows that the component profile remains remarkably unchanged in the wax ester envelope (Figure 2), resembling the original lanolin substrate in this case. However, additional lower molecular weight peaks are apparent in comparing the profile with the original cosmetic grade lanolin, particularly the growth of a peak close to cholesterol which has been identified as cholesterol acetate.

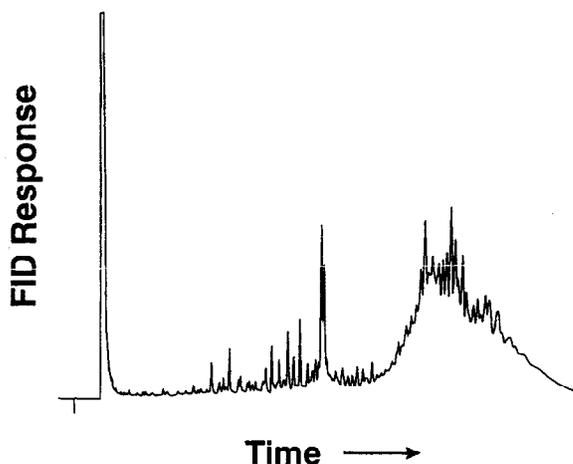


Figure 2. Capillary SFC separation of acetylated lanolin sample (SB-octyl, program 1).

Saponification of lanolin yields over 70 components consisting of acids and alcohols, the product mixtures having average molecular weights in excess of 300 daltons. The acids consist of several structural types: *n*-acids, anteiso-acids, iso-acids, and alpha-hydroxy acids (ranging in carbon number from 7 to 40) [27], all in low percentages as judged from their distribution in the chromatogram in Figure 3. Their partial resolution suggests that the acids are of sufficient chain length to undergo dispersion interaction with the nonpolar bonded polymeric stationary phase, SB-octyl. The polarity of these acid moieties substantially reduces their retention time on a standard nonpolar column such as the SB-octyl, and it is apparent that this stationary phase is the wrong choice for totally resolving the acid products from the saponification of lanolin. Application of differ-

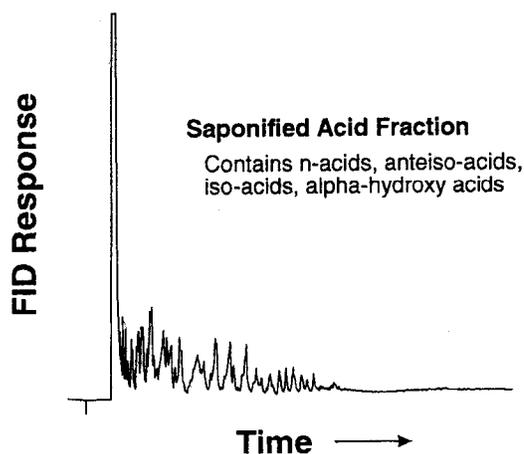


Figure 3. Capillary SFC separation of acid fraction derived from saponification of lanolin (SB-octyl, program 1).

ent programs to provide better resolution of the lanolin acid fraction on a common column did not substantially change the overall retention pattern of the lanolin acid chromatogram. Interestingly, application of a more polar bonded phase (SB-cyano-propyl-50) did not change the resolution of these acids appreciably or their retention indices. It was hoped that application of the SB-smectic phase might resolve some of the moieties in the complex lanolin mixtures based on their spatial dimensions. However, for this application this column proved inadequate and yielded very low resolution and plate counts.

The alcohol fraction from the lanolin saponification is shown in Figure 4. It is less complex than the profile obtained for the acid moieties, and the SB-octyl column resolves several major components to permit their identification. Apparent are the presence of the major alcohols, cholesterol as well as lanosterol and agnosterol [28]. These major sterol constituents were identified by comparing their retention time with those obtained by injecting available commercial standards of the pure sterols, as well as comparison with compositional data from a gas chromatography (GC) analysis of this fraction.

To complete the chromatographic profiling of lanolin and its chemical derivatives, a sample of hydrogenated lanolin was run under identical conditions (program 1, SB-octyl column) to yield the result shown in Figure 5. Hydrogenation of lanolin, accomplished at high pressures (5000 psi), produces a complex mixture of alcohols and some hydrocarbons of lower molecular weight than lanolin, as judged from the retention times of alcohols versus the components initially in the lanolin profile (see Figure 5). A small residual of unreacted lanolin is

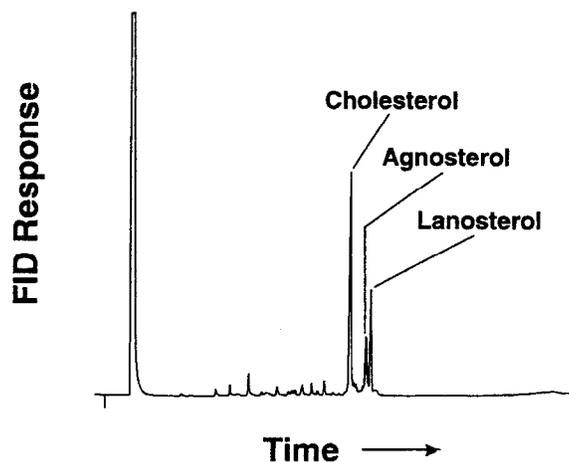
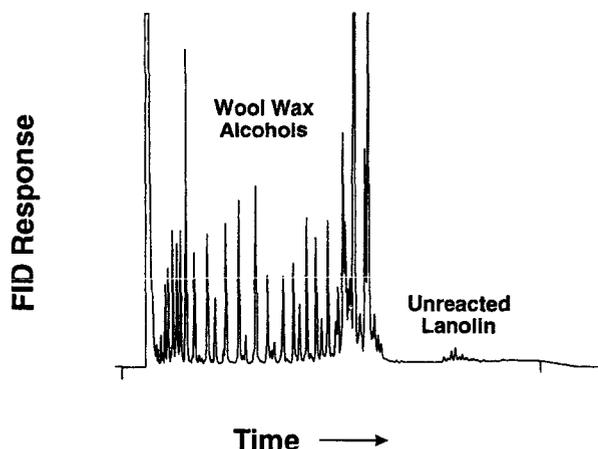


Figure 4. Capillary SFC separation of alcohol fraction derived from saponification of lanolin (SB-octyl, program 1).



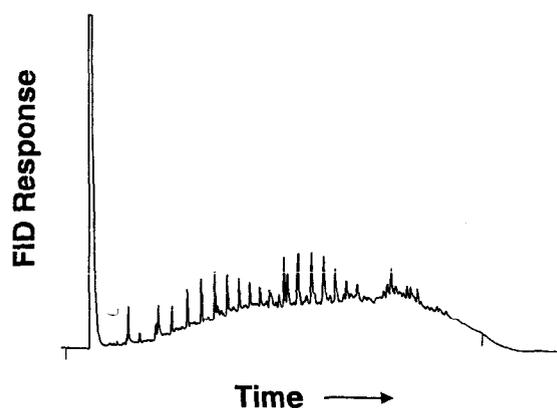
**Figure 5.** Capillary SFC separation of hydrogenated lanolin mixture (SB-octyl, program 1).

apparent in the SFC profile indicating that a high conversion had been achieved of the lanolin to the final product. This confirms the fact that hydrogenated lanolin has a very low saponification value of only 3–4 [29].

The chromatograms in Figures 1–5 permit identification of the various lanolin derivatives based upon the “polarity” of the solutes contained within the various lanolin derivatives. By using the SB-octyl as a standard column, it can generally be concluded that many of the reacted lanolin mixtures contain components of lower molecular weight than the starting lanolin profile and that they are nonpolar moieties. This is particularly apparent when one attempts to use the SB-cyanopropyl 50 column on these mixtures of lanolin derivatives; in general, there was a substantial reduction in retention times resulting in peak overlap after elution of the solvent peak in the chromatograms. This verifies the importance of column chemistry in achieving adequate resolution of hydrophobic solutes that comprise lanolin and its various derivatives.

It is extremely interesting to compare the SFC profile of a “synthetic” lanolin with those that are characteristic of “natural” lanolins, as shown in Figures 1(a)–(c). Figure 6 is an SFC profile of a commercial synthetic lanolin sample using the same chromatographic conditions as noted for the natural lanolin in Figure 1(c). It is apparent from the profile that there is no cluster of higher molecular weight peaks representing the wax ester components but that the synthetic lanolin “analogue” consists of discrete components of lower molecular weight relative to natural lanolin.

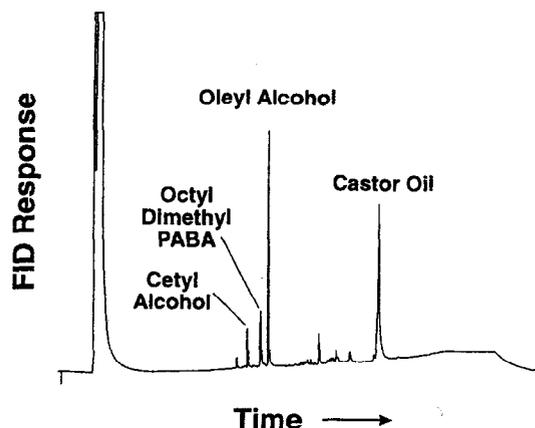
**Cosmetic deformulation.** Capillary SFC can be an extremely valuable technique in deformulating cosmetic products containing a large number of non-



**Figure 6.** Capillary SFC separation of components in synthetic lanolin sample (SB-octyl, program 1).

polar, hydrophobic components or submixtures. We have found that by directly dissolving the cosmetic samples in *n*-hexane or methylene chloride one can directly inject the sample onto the SFC column and commence the programmed run without further sample preparation. This method is of course limited to the detection of cosmetic ingredients that will dissolve in the SC-CO<sub>2</sub> mobile phase as well as the solvents used in preparing the injection solution. Despite this limitation, valuable qualitative information can be ascertained using the SFC approach.

As an example of this method, Figure 7 is the SFC profile of the components in a “quencher” lipstick. The components in the profile were identified by using analytical standards of the pure solutes in conjunction with the stated label contents on the commercial product. Two long-chained, hydrophobic alcohols were identified in the profile: cetyl and oleyl alcohol. Also confirmed was the presence of a preservative, octyl dimethyl PABA, referred to as Padimate O in some cosmetic formulations [30].



**Figure 7.** Capillary SFC separation of components in a quencher lipstick formulation (SB-octyl, program 2).

This is the ester formed between octyl alcohol and dimethyl *p*-amino-benzoic acid. Finally, the presence of castor oil in the formulation was also verified by a comparison of the elution position of the oil with an analytical standard (castor oil is predominately a ricinoleic acid-based triglyceride). This points out one of the advantages of capillary SFC, i.e., for surveying for the presence of relative nonpolar components in a formulation.

Figures 8–10 are the SFC profiles of various commercial lip balms. These chromatograms as well as that provided in Figure 7 were generated using program 2 as given in Table II. As can be seen from inspection of the chromatograms, each lip balm formulation has several components in common: cetyl alcohol, padimate O, and petrolatum. However, there are other distinguishing features which set the various lip balms apart. For example, two of the three balms contain camphor as an active medicinal ingredient (see Figures 8 and 10). Only one formulation

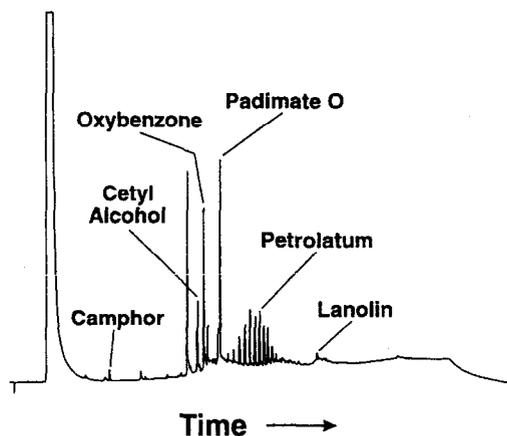


Figure 10. Capillary SFC separation of components in a blistek lip balm (SB-octyl, program 2).

shows the presence of a lipophilic ester, isopropyl myristate, a common cosmetic ingredient (Figure 9). Finally, Figure 10, which has perhaps the most complex profile, includes the presence of oxybenzone (2-hydroxy-4-methoxybenzophenone), a sun screen, and a trace of lanolin, the latter being identified by injecting a more concentrated sample and comparing it with standard lanolin profiles such as shown in Figures 1(a)–(c). In this way, SFC can be used to distinguish the components in various commercial products and, at the same time, can provide valuable information to the formulator concerned with matching a competitive product.

In summary, capillary SFC can be used to establish signature profiles for many natural as well as synthetic cosmetic ingredients. These include such components as a jojoba oil, the common vegetable oils [31], lanolin, medicinal ingredients, and a host of synthetic stabilizing additives. The described methods are also amenable to characterizing chemical derivatives of such components as lanolin or jojoba, due to the ability to fractionate a wide molecular weight range of both reactant and products. Optimum resolution of such species is attained on nonpolar stationary phases for the commercial products that we have characterized to date. Finally, once the above retention characteristics have been established, the capillary SFC method can be of considerable assistance in de-formulating commercial cosmetic products containing components that are miscible with the mobile phase densities quoted in this study.

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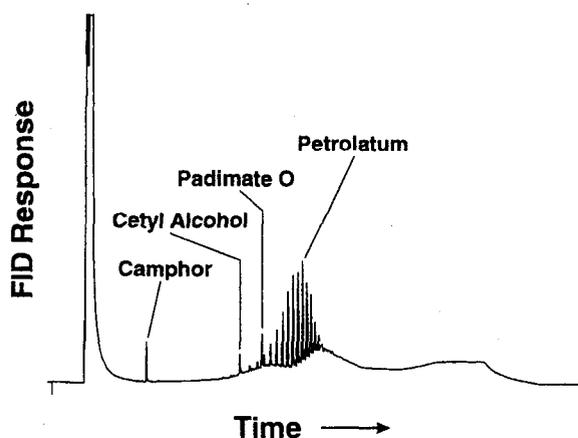


Figure 8. Capillary SFC separation of components in a RX lip balm (SB-octyl, program 2).

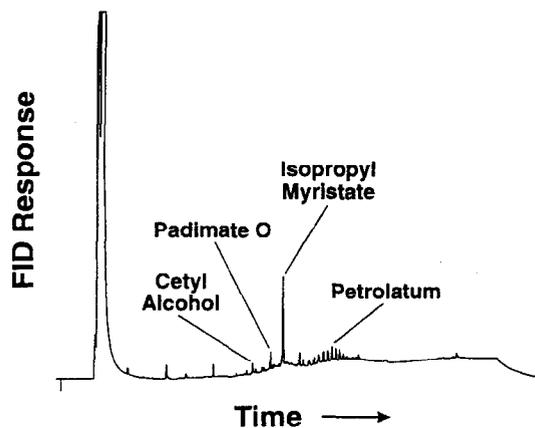


Figure 9. Capillary SFC separation of components in a chapstick lip balm (SB-octyl, program 2).

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