

I INTRODUCTION

Nowadays, the potential risks regarding cosmetic products are closely controlled by the European Union (EU). Indeed, the presence of certain natural compounds in cosmetic products is limited due to health and safety reasons. One of the recent restrictions concerns the concentration of furocoumarins in cosmetics. These photosensitizing compounds are present in citrus oils used by perfumers in the fragrance industry. Moreover, a variety of compounds (terpenes, coumarins, flavonoids) can be found in these essential oils.

The separation and quantification of furocoumarins is achieved with a reference method by RP-HPLC (C18 phase), using a complex multi-step gradient (water-acetonitrile-methanol-tetrahydrofuran) lasting about 55 minutes.

Our goal was to develop a shorter and environmentally more friendly (=green) separation method by SFC. For this purpose, the effects of numerous analytical parameters were investigated: nature of stationary phase, flow rate, modifier percentage and temperature.

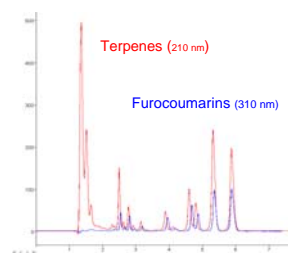
II COLUMN SELECTION

Uptisphere ODB, C18, Interchim
Synergi Fusion RP, C18 hydrophilic endcapping, Phenomenex
Altima HP AQ, C18 hydrophilic endcapping, Grace-Alltech
Acclaim Polar Advantage, C18 with sulfonamide embedded group, Dionex
Acclaim Polar Advantage II, C18 with amide embedded group, Dionex
Silica gel, Kromasil, Eka nobel
Cyano, Princeton Chromatography
2-Ethylpyridine, Princeton Chromatography
Synergi polar RP, Phenylxypropyl, Phenomenex
Cholester, Cosmosil Cholester, Nacal Tesque
Discovery HS F5, Pentafluorophenyl, Supelco
Gemini phenyl-hexyl, Phenomenex
Pursuit Diphenyl XRs, Varian
Cogent C18, type C silica, Microsolv Technologies

Different qualities of separations were obtained on the phases listed in the box.

Aromatic or polar embedded groups seem to favour separation, whereas polar phases favour class fractionation.

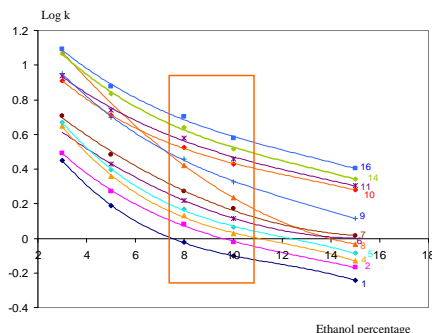
Finally, the Discovery HS-F5 (pentafluorophenyl) phase was selected because of its ability to separate furocoumarins from terpenes, and to provide the cleanest chromatogram in screening conditions.



III EFFECT of ETHANOL PERCENTAGE

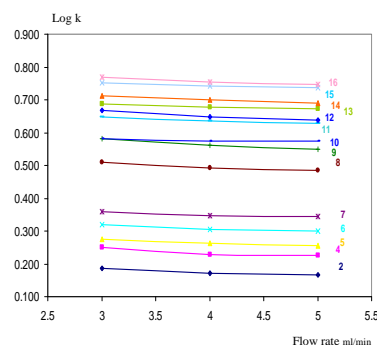
In order to provide green separation conditions, ethanol was selected as modifier. The ethanol addition, from 3 to 15 % induces a decrease in retention as expected. Variations in retention depend on the chemical structure of the compounds. For instance, compounds 1, 4 and 5 are all epoxides, while compounds 8 and 9 have two hydroxyl groups thus show larger retention variations.

To achieve complete separation, the modifier percentage should be 8-10%.
Other conditions : T = 25°C; P = 15 MPa; F = 3 ml/min.



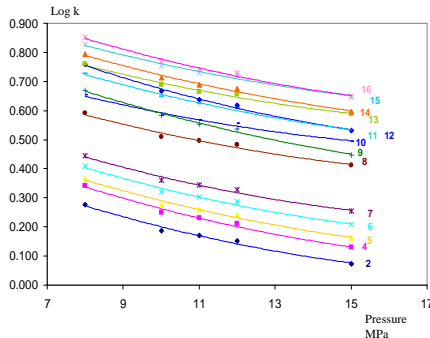
IV EFFECT of FLOW RATE

The flow rate increase reduces retention of compounds, due to density changes of the mobile phase. Separation factor (α) of several compounds (9/10; 11/12) can be modified. Flow rate may be checked as a minor parameter influencing retention and separation.



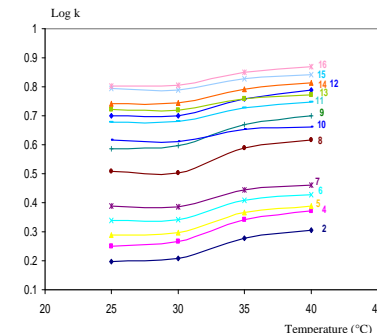
V EFFECT of OUTLET PRESSURE

The outlet pressure was varied from 8 to 15 MPa. Its increase causes a decrease in retention and modifies separation factors (α) for the same couples of compounds as in the flow rate study (9/10; 11/12).



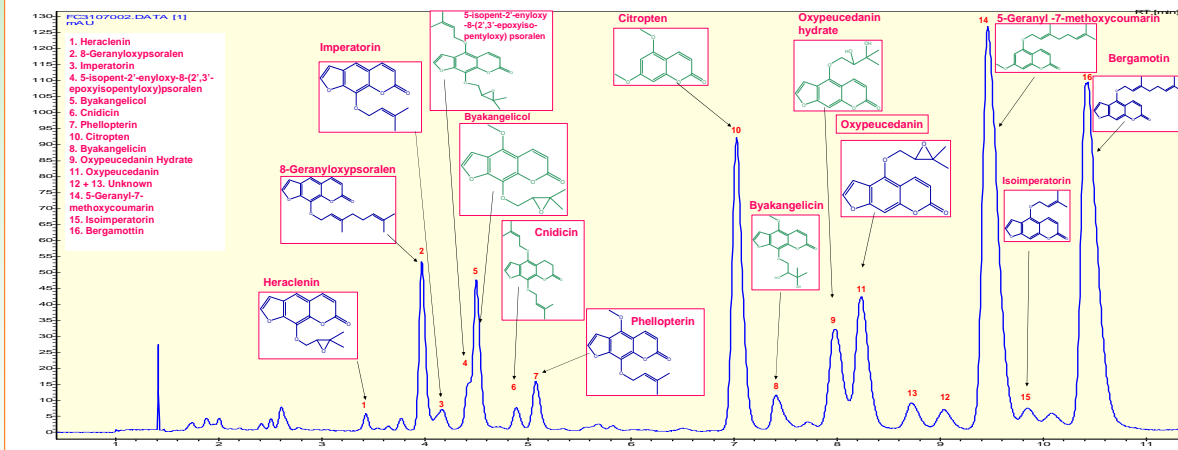
VI EFFECT of TEMPERATURE (8 MPa; 9% EtOH; 3 ml/min)

Temperature was modified from 25 to 40°C. The temperature increase favours retention. Density changes are responsible for this behaviour. Once again, retention variations of compounds 8 and 9 are the largest. One can expect this to be due to variation of the amount of ethanol adsorbed onto the stationary phase induced by temperature increase. Compound 12, which is still unidentified, follows a close retention behavior, indicating that it might also possess hydroxyl groups.



VII FINAL CONDITIONS

The separation of Lemon residue was carried out at 35°C, 10 MPa; 3 ml/min, with a mobile phase gradient of EtOH in CO₂: 7% during 4 min; 8% during 3.2 min; 9% during 4 minutes. The change of modifier percentage took one minutes between two steps.



The separation was achieved in less than 11 minutes with SFC, instead of 55 minutes by HPLC. Most of the main components of Lemon residue were identified by standard analyses (formula in blue), or by NMR (formula in green). The compound structures and the chromatographic behaviours induced by parameter changes are in accordance. This can be helpful to obtain information on the chemical functional groups of the compounds: e.g. epoxy, hydroxyl. The position of substituents (5 or 8) was also found to play an important role in chromatographic behaviour.

The described green separation technique (CO₂/EtOH) is applicable to other essential oil analyses.

The speed of analyses in SFC allows quickly to check the stationary phases available in the laboratory. On the other hand, the use of LSER characterisation could be helpful to reach a satisfactory varied choice of stationary phases (1).