CHAPTER 7

CONCLUSION

7.1 Supercritical fluid chromatography analysis

Chemical class separation with SFC produces very important information. With a silica-gel PLOT column, four essential oil samples (*Tagetes minuta, Artemisia afra, Cymbopogon citratus & flexuosus*, and *Pelargonium capitatum*) were separated into different chemical classes without the need to use either modifier or the backflush method. Terpene hydrocarbons are well separated from the oxygenated compounds. Oxygenates are partially separated into esters, ketones, aldehydes and alcohols.

The CO_2 mobile phase in SFC is compatible with the FID quantification of the separated essential oil groups and results about the relative amount of each eluted fraction were obtained. With SFC it is possible to separate samples at room temperature, near the critical temperature of the CO_2 mobile phase, to enhance the group separation. Operational conditions for SFC separation were obtained to separate the essential oils samples. It was found that near critical temperature (28 ^{o}C), 110 atm pressure and a flow rate of 480 ml/min (7.7 cm.s^{-1}) and split of 50 ml/min to SFC-FID and 430 ml/min to fast GC gave fair group-type separation of essential oil samples especially for separating terpene hydrocarbons from the oxygenates. Volume flow rates apply to measurements of CO_2 flow after expansion to atmospheric pressure.

7.2 Comprehensive Two-dimensional SFCxGC analysis

SFC-FID on its own provides useful information about the relative percentages of some chemical classes contained in essential oil samples. To increase the amount of data that can be obtained from the sample mixtures, additional analysis of the SFC groups is required. Comprehensive coupling of SFC with fast temperature programmed gas chromatography by a flow modulator using a stopped-flow arrangement was used to separate four samples of essential oils. The separated peaks obtained from essential oils by SFC contained many components. Some of those co-eluting compounds are individually of interest to the flavour and fragrance industries. A measurement of individual components is often necessary. With fast temperature programmed GC, information regarding individual components contained in each class was obtained by effecting boiling point separation of the groups. The terpene hydrocarbons, separated from the oxygenates in SFC, is further separated into C10 monoterpenes, C15 sesquiterpenes and C20 diterpenes in some of the oils.

With favourable temperature conditions both in GC (temperature ramp) and SFC (near critical temperature of CO_2 mobile phase) different groups of esters were distinguished with SFCxGC. This feature was observed in a chromatogram of *Pelaragonium* (figure 6.5) where ester formates (C11), ester acetates (C12), ester propionates (C13), ester butyrates (C14) and tiglates (C14) were well differentiated. This demonstrates some of the SFCxGC instrument capabilities. SFCxGC shows an increased separation power over SFC one-dimensional separation of essential oils. This increased separation power is a result of the SFCxGC advantages which include the: increased peak capacity, sensitivity, and ordered separations based on chemical class.

By using the distinctive peak patterns observed in all four oils, it was possible to compare the oils and characteristic features were observed such as the presence of certain compounds in some of the oils and their absence in others. With the literature information on the composition of the oil and the use of standards it is possible to identify some of the major components in the oils. The possibility of obtaining the

three-dimensional chromatograms allows easy visualization of the oil components including the lower concentration peaks. The quality of the SFCxGC chromatogram was found to be dependent on the reproducibility of the fast GC that is influenced by the thermocouple placement on the column. GC reproducibility was found to be better than SFC reproducibility in consecutive SFCxGC runs. The SFC retention variability seems to be caused by (1) variation in the linear velocity of the mobile phase and (2) insufficient temperature control of the SFC column at the relatively low temperature (28 ^{o}C) by the GC oven.

The power of SFCxGC for fingerprinting of essential oils was illustrated by differentiating samples of *C. citratus* oil obtained from different geographical locations. Samples of *C. flexuosus* were also readily distinguishable from *C. citratus*.

Although SFCxGC is not faster than normal capillary GC, it should be appreciated that much more information can be obtained in the same time. A fingerprint pattern containing information on essential oils composition (especially chemical group composition) can be obtained. This information can be used for quality control purposes or identification of unknown essential oil sample mixtures by using pattern recognition. The opportunity to apply mass spectrometry to the second dimension separation would tremendously enhance the qualitative power of SFCxGC for compound identification. Without mass spectrometery, no identification of unknown components can be achieved, however, valuable information as to the chemical class and volatility of unknowns can be achieved. Fortunately, conventional MS scan speeds of 10 scans per second can effectively deal with the fast GC peaks of 0.5 sec width.

7.3 Possible future research work on SFCxGC system.

1. Additional work on representing a two-dimensional SFCxGC chromatogram is required that includes a bar polarity scale and integration of the Kovats Index scale to the fast GC retention axis.

2. The stability of the CO_2 pump flow and the SFC column temperature must be improved to alleviate the retention times shifts explained in the polarity separation.

3. Although the in-house built resistively heated fast GC shows acceptable retention time stability, this needs further attention to improve the appearance of the three dimensional peaks, also to allow automatic integration of these peaks for quantitative analysis. A reliable means of attaching the micro-thermocouple to the column or an alternative fast temperature probe is a prerequisite for improved GC retention time stability.

4. Mass spectrometry should be coupled in-line with the SFCxGC to alleviate the problem of peak allocation in known mixtures and to identify unknown components.