

**Characterization of essential oils by comprehensively coupled
supercritical fluid and gas chromatography
(SFCxGC)**

BY

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SUMMARY

Essential oils are amongst the most complex samples an analyst can face in terms of the number of compounds involved. In many cases, minor components are of interest as they can impart a distinctive fragrance character to the oil. Because of the closely related structures and molecular weights among terpenes, positive identification of individual compounds is very difficult with a single chromatographic technique. Further, most of the analytical information is lost when a single technique is used because of the limited peak capacity and the resulting peak overlap. For many years, gas chromatography coupled to mass spectrometry (GC-MS) has been the benchmark

technique for qualitative and quantitative analysis of essential oils. Retention indices and mass spectra have to be used in combination for confirmation of the identity of components in an essential oil. Other multidimensional or hyphenated techniques also offer advantages that aid in the identification of essential oil components. This thesis demonstrates the application of a comprehensively coupled supercritical fluid and fast temperature programmed gas chromatograph (SFCxGC) to the analysis of essential oils. An SFCxGC instrument was used to analyse the essential oils of *Cymbopogon* (lemongrass), *Artemisia afra* (wilde als), *Tagetes minuta* (kakiebos) and *Pelargonium* (geranium) species. The unique application of a porous layer open-tubular (PLOT) column, used in conjunction with supercritical carbon dioxide is demonstrated to effect group separation of polar, oxygenated compounds. This separation and elution of very polar compounds from a silica gel column is believed to occur due to the reduced phase ratio (β) of the system obtained by increasing the volume available to the mobile phase compared to that of a packed column. This separation obtained in the SFC is used to separate essential oils into different chemical classes such as non-polars, ethers, alcohols. Separated chemical classes are re-injected on-line by use of a modulator into a fast, second dimension, temperature programmed GC to effect separation of individual compounds based on their volatility. The entire sample is analysed by both the SFC and GC in such a way that the resolution obtained in the first dimension is conserved by the GC analyses. By using a range of standards, some of the peaks in these oils could be assigned. The identification of compounds was greatly aided by the combination of the two separation dimensions. The comprehensive two-dimensional technique arranges component peaks in a plane from which chemical class and volatility information of each component is readily obtained. The elution pattern within the two-dimensional chromatograms may also be used for direct comparison of oils without identification of the components in the essential oils.

**Die karakterisering van essensiele oliës met omvattend-gekoppelde
superkritiese-fluïed-en gas chromatografie
(SFCxGC)**

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Samevatting

Vlugtige plantolies is van die mees komplekse monsters waarmee 'n analis te doen kan kry wat betref die aantal verbindings betrokke. In baie gevalle is lae-voorkoms komponente van belang aangesien hulle 'n karakteristieke geur kan verleen aan die olie. As gevolg van die nou-verwante strukture en molekulêre massas van terpene, is positiewe identifikasie van komponente baie moeilik met 'n enkele chromatografiese tegniek. Verder is meeste van die analitiese inligting onbekombaar met 'n enkele tegniek, as gevolg van beperkte piek-kapasiteit en die gevolglike oorvleueling van

komponente. Gaschromatografie en massaspektrometrie was vir baie jare die staatmakertegnieke vir die kwalitatiewe en kwantitatiewe ontleding van vlugtige olies. Gewoonlik moet retensie-indekse en massaspektra saam gebruik word (GC-MS) vir bevestiging van die samestelling van 'n vlugtige olie. 'n Superkritiese-vloeistofchromatograaf, omvattend gekombineer met 'n vinnige temperatuur-geprogrammeerde gaschromatograaf (SFCxGC) word gebruik om vlugtige plantolies te ontleed uit die volgende plante: *Cymbopogon* (sitroengras), *Artemesia afra* (wilde-als), *Tagetes minuta* (kakiebos) en *Pelargonium* (malva). 'n Poreuse-laag, oop-buis-kolom (PLOT-kolom) met 'n klein faseverhouding (β) word in die SFC-dimensie gebruik om die monster te skei in verskillende chemiese klasse. Daarna word die geskeide klasse direk deur middel van 'n modulator ingelaat in 'n vinnige, tweede-dimensie temperatuur-geprogrammeerde gaschromatograaf om individuele komponente te skei op grond van hul vlugtigheid. Deur die gebruik van 'n reeks standaarde kan sommige van die komponente geïdentifiseer word. Vanuit ons kennis van die hoofkomponente in hierdie olies en hul retensie-gedrag, kan die meeste pieke uitgeken word met die twee-dimensionele SFCxGC chromatogramme. Dit blyk dat daar 'n elueringspatroon is in die twee-dimensionele chromatogramme wat gebruik kan word om olies te vergelyk en om onbekende stowwe in vlugtige plantolies uit te ken.

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ABBREVIATIONS

FID	:	Flame Ionization Detector
GC	:	Gas Chromatography
GCxGC	:	Comprehensive two-dimensional gas chromatography
GC-MS	:	Gas Chromatography-Mass Spectrometry
GC-TOF-MS	:	Gas Chromatography-Time-of Flight- Mass Spectrometry
HETP	:	Height Equivalent of the Theoretical Plate
LCxLC	:	Comprehensive two-dimensional liquid chromatography
MS	:	Mass spectrometry
PLOT	:	Porous Layer Open-Tubular
SFC	:	Supercritical fluid chromatography
SFCxGC	:	Comprehensive two-dimensional supercritical fluid and gas chromatography
SPME	:	Solid-Phase Microextraction
SMO	:	Statistical Model of component Overlap
LRI	:	Linear Retention Index

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