CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Essential oils are amongst the most complex mixtures an analyst can face in terms of the number of compounds involved. An estimated 1,200 compounds, including terpenes and their corresponding aldehydes, ketones, alcohols, phenylpropanoids, hydrocarbons, esters, oxides and sulfur compounds, have been identified in essential oils[1]. Essential oils are defined as volatile plant products whose constituents are a complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, ketones, esters and alcohols. They contain volatile compounds of plant origin with unique properties that have been prized worldwide for thousands of years.

Because of the enormous amount of plant material required to produce natural essential oils, products in the market are often adulterated with lower quality, commercial grade oils or synthetic compounds. These reduce the costs in order to increase the profit margin, a fact not usually revealed on the label. Issues concerning essential oil adulteration and the dilution of the original oils with those of lesser value have also been investigated[2,3].

Oils from thousands of plant species have been extracted and are commercially available[4,5]. Essential oils are extracted from plant materials by a number of procedures including steam distillation, vacuum distillation, solvent extraction, cold-pressing and hot-pressing[6]. The composition of these oils can vary significantly with place of origin, harvest season, and climate. A common feature is that essential oils possess the essence of some plant, the identifiable aroma, flavour or other characteristic that has some practical use. They are used in cosmetics (perfumes), food flavours, deodorants, pharmaceuticals and embalming antiseptics.

Because of the incredible complexity and hundreds of different chemical constituents contained in one single oil, it becomes clear that analysis of essential oils is difficult. To add to the complexity of the volatile oils, the time of harvest, climate, the soil and the mode of essential oil extraction influences the oil composition and consequently the amount of biologically active substances[7]**.** The quality and price of some oils are based on the percentage content of some components contained in the oil, so separation and measurement of these components are very important. This is usually done using chromatography and spectroscopy. Gas Chromatography (GC) and GC coupled to mass spectrometric detection (GC-MS) has been used to ascertain quality and purity of most essential oils[8,9].

Recently, the greatest efforts have been directed towards improving methods in order to obtain better separation, especially for complex samples, at lower cost and faster speed. The identification of components based on only one parameter, typically retention time, has become inadequate for complex mixtures. A different strategy for achieving unequivocal identification of compounds, is to increase the number of parameters that can be used simultaneously in detection[10].

The use of the linear retention indices (LRI) and mass spectra data for essential oil compositional analysis was developed into an index[11] that combines the two criteria for final identification of the compounds. The use of either mass spectral results or LRI alone may lead to erroneous results.

A difference in mass spectra may be observed if the spectra were obtained using an ion trap MS[12]. Often different spectra are reported in the MS library for one component peak. The spectral similarity of a great number of essential oil components often precludes positive identification of individual components. Mass spectra for many sesquiterpenes are identical or nearly the same[13]. It has been found that more than 230 naturally occuring sesquiterpenes have a molecular mass of 204[11]. Since many of these sesquiterpenes may be present in the same essential oil it is very difficult if not impossible to positively separate and identify all components in an oil with a one-dimensional technique. Compilations such as that of Adams[5] suggest standard conditions that other researchers may use to identify the chromatographic peaks in the chromatogram.

Multidimensional techniques offer a solution to this problem by providing more resolving power, resulting in enhanced peak capacity, selectivity and a larger number of parameters for the characterisation and identification of components in complex mixtures[10]. In many cases the dimensions are two or more chromatographic steps, or a chromatographic separation with spectroscopic detection.

Multidimensional liquid chromatography-gas chromatography (LC-GC), in which an HPLC is coupled on-line with GC, gives lots of information concerning a sample in a single run. Therefore, multidimensional HPLC-GC is useful for the analysis of complex samples, such as natural products (essential oils)[14,15]. HPLC offers chemical class separation of compounds. One of the problems in the HPLC-GC system is the large volumes of the HPLC mobile phase that need to be removed when introduced into the GC injector[16]. Pre-treatment or clean-up of samples is important, before injection into the analytical system. This can be labour-intensive and time consuming. Therefore, there is a high demand for on-line systems that can do pre-separation of complex mixtures into groups, thus reducing the complexity of the sample matrix before detailed on-line analysis of individual compounds in each group to yield valuable information in a short time.

Terpenic hydrocarbons are unstable to heat and light and they degrade to produce compounds with undesirable off-flavours when exposed to light or heat for long a time. Furthermore, terpene hydrocarbons do not contribute much to the flavour or fragrance of the oil, even at higher concentrations[13]. The oxygenated compounds such as aldehydes, esters and alcohols determine the characteristic flavour and odour of essential oils so valuable for their applications in a number of industries. These oxygenated groups are difficult to elute on silica- gel due to large retention factors when using the fairly non-polar CO_2 as mobile phase only. However, with SFC using a porous layer open tubular (PLOT) column, oxygenated compounds are eluted[17].

In South Africa, *Cymbopogon citratus & flexuosus* (lemongrass), *Tagetes minuta* (kakiebos), *Artemisia afra* (wilde als) and *Pelargonium* (geranium) plants, among others, are grown for commercial production of essential oils. Analysis of these oils is important to the farmers for a number of reasons, for example, quality control purposes. Bioprospecting for new oils in indigenous plants is also of interest. Therefore, there is a need for improved analytical techniques that would provide valuable information at lower cost and in a reduced time.

1.2 Approach

The main purpose of the study is to investigate the potential of SFCxGC in fingerprinting essential oils. A successful fingerprinting technique should produce sufficient, reproducible information to discriminate between oils of closely related species. It should also discriminate between oils from the same species of different geographical origin. This technique provides a new solution for the analysis of complex essential oils by increasing the selectivity of the separation method and reducing peak overlap.

In SFCxGC, mixtures of compounds are subjected to two independent separation dimensions. In the first dimension compounds are separated into different chemical classes using SFC with supercritical carbon dioxide as mobile phase. The separated groups are further separated into individual compounds in the fast temperature programmed GC based on their different boiling points. The combination of the two parameters (polarity and volatility) aids assignment of compound identity.

Supercritical fluid chromatography (SFC) is a separation technique that bridges GC and LC. SFC uses the chemical class separation capability of normal phase liquid chromatography. The availability of gas chromatographic flame ionization detector (FID) simplifies the use of SFC as compared to LC especially for compounds without a chromophor. The use of SFC for group separation of essential oils has been reported[18,19].

A comprehensive two-dimensional SFCxGC system provides a substantial increase in peak capacity by serially coupling two separation mechanisms for the analysis of natural products (essential oils). A flow modulator (using the stop-flow principle) interfaces the SFC to the fast temperature- programmed GC, facilitating the on-line transfer of eluents from the SFC into the fast GC. The modulator cuts the entire sample stream from the SFC into consecutive slices and re-injects them into a fast second dimension for further analysis. The resulting two-dimensional chromatograms provide information relating to both the chemical class and volatility of the components in a sample and greatly aids the identification of unknown compounds in complex mixtures.

1.3 Presentation and arrangement

Each chapter deals with a separate aspect of the research and has its own references (found at the end of each chapter).

Chapter 2 explains the production of essential oils and methods of isolation, followed by a brief discussion of essential oil plant material studied in this work.

Chapter 3 is devoted to methods for essential oils analysis. Starting from one-dimensional analysis to coupled techniques, including comprehensively coupled techniques. The fundamental concepts of multidimensional chromatography and fast GC are also introduced. This will allow better understanding of the theory and operational procedures of comprehensive two-dimensional supercritical fluid and fast temperature programmed gas chromatograph (SFCxGC).

Chapter 4 looks at theoretical consideration and physico-chemical properties of supercritical fluids as mobile phases in chromatography.

Chapter 5 covers the instrumental aspects of SFC and optimisation of the system parameters (mobile phase flow rates, temperature, and pressure) for group-type separation of essential oils. It also suggests how the unique elution and separation of the PLOT column can be explained.

Chapter 6 describes in detail the instrumentation of comprehensive two-dimensional SFCxGC. Problems experienced with the system are discussed, as well as the potential of the SFCxGC for the analysis of complex mixture such as essential oils.

Chapter 7 provides the conclusion to the project.

1.4 References

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