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Review

Gas chromatographic technologies for the analysis of essential oils

Philip J. Marriott[a,*], Robert Shellie[a], Charles Cornwell[b]

[a]*Chromatography and Molecular Separations Group, RMIT University, Department of Applied Chemistry, GPO Box 2476V, Melbourne 3001, Australia*

[b]*Australian Botanical Products, 39 Melverton Drive, Hallam 3803, Australia*

Abstract

Essential oil analysis has basically had one technical goal: to achieve the best possible separation performance by using the most effective, available technology of the day. The result achieved from this may then be used to answer the research or industrial analysis questions which necessitated the analysis. This may be for comparative purposes, where one oil is contrasted with other(s) for quality control or investigation of adulteration, to discover new components, or to characterise the chemical classes of compounds present. Clearly, today the analyst turns to chromatography as the provider of separation and then may supplement that with mass spectrometry to aid identification. The power of GC–MS means that advances in both the separation technique, and improvements in mass spectrometry detection – along with improved data handling tools – will immediately be relevant to the essential oil area. This present review outlines the developmental nature of instrumental approaches to essential oil analysis using gas chromatography. Mass spectrometry will be included to the extent that it represents the hyphenation of choice for most analysts when analysing essential oils. Thus single-column and multi-dimensional analysis will be covered, as will sample handling or introduction techniques prior to the analysis step, where these techniques provide some measure of separation. The recent demonstration of comprehensive gas chromatography will be discussed as the potentially most powerful separation method for essential oils. This brief review is not intended to be a comprehensive dissertation on the field of essential oil analysis since that would require sufficient space to occupy a book in its own right. Rather, it will outline selected considerations and developments, to help explain where new technology has been applied to advantage in this field. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Gas chromatography, comprehensive two-dimensional; Multidimensional separation; Sample introduction; Essential oils

Contents

*Corresponding author. Tel.: +61-3-9925-2632; fax: +61-3-9639-1321.
E-mail address: philip.marriott@rmit.edu.au (P.J. Marriott).

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1. Introduction and gas chromatography techniques

Whilst gas chromatography (GC) need not be described in detail here, a brief comment on the suitability of GC for the essential oil area of analysis to be discussed is warranted. Gas chromatography has a molecular mass operating range from 2 (molecular hydrogen) to about 1500 mass units. Whilst there does not seem to be an International ''league table'' of the heaviest mass compounds analysed, in terms of normal alkanes specialist analyses might report successful chromatography up to C_{100} *n*-alkane (about mass 1400). Derivatised porphyrins have been reported up to about 1200 mass units, with their spherical nature leading to retention indices of 4000 (thus eluting with similar retention factor as C_{40} *n*-alkane which has a mass of 562). Within this mass range, suitable compounds which can be chromatographed will be classified as permanent gases (i.e., highly volatile), volatile compounds (up to maybe a mass of 200 mass units) and semi-volatile compounds (those of higher mass). By their nature, essential oils will range from volatile through to semi-volatile compounds. Being derived from natural flora, they will serve as highly volatile alarm-type compounds, which must rapidly diffuse into the surrounding air, through to more waxy leaf compounds, which have a lesser vapour pressure and provide part of the structural constituents of a plant (e.g., membrane or cell composition). There will be many components of the leaf or plant woody tissue that are non-volatile (e.g., cellulose) but they need not concern this review.

The compounds of concern to this study will therefore range from highly volatile through to about mass of 400; it will largely consider the terpenoid compounds. This range is particularly suited to gas chromatographic analysis. The only other consideration will be that the compounds must survive their passage through the heated injector and chromatography column. Whilst some compounds may be prone to thermal alteration, the majority will largely remain intact. Terpenes, of which the essential oil terpenes are a sub-category, derive from the head-to-tail linkage of the ''isoprene'' moiety (the C_5 compound, 2-methyl-1,3-butadiene), and have carbon ranges from C_{10} to C_{40}. The ''terpene'' nomenclature of these compounds is shown in Table 1.

Examples of selected compounds representing these terpenes are illustrated in Fig. 1.

Discussion of precursor biological compounds (e.g., isopentenyl pyrophosphate) and biosynthetic pathways leading to essential oil generation will be out of the scope of this review.

There are two primary considerations which must be taken cognisance of when discussing the analytical separation of essential oils. Firstly, recognising that chromatographic methods – primarily gas chromatography – will be the most appropriate analytical instrumental approach, one must decide

Table 1
Terpene nomenclature for isoprenes

Carbon atoms	Isoprene units	Nomenclature
10	2	Monoterpenes
15	3	Sesquiterpenes
20	4	Diterpenes
25	5	Sesterterpenes
30	6	Triterpenes
40	8	Tetraterpenes

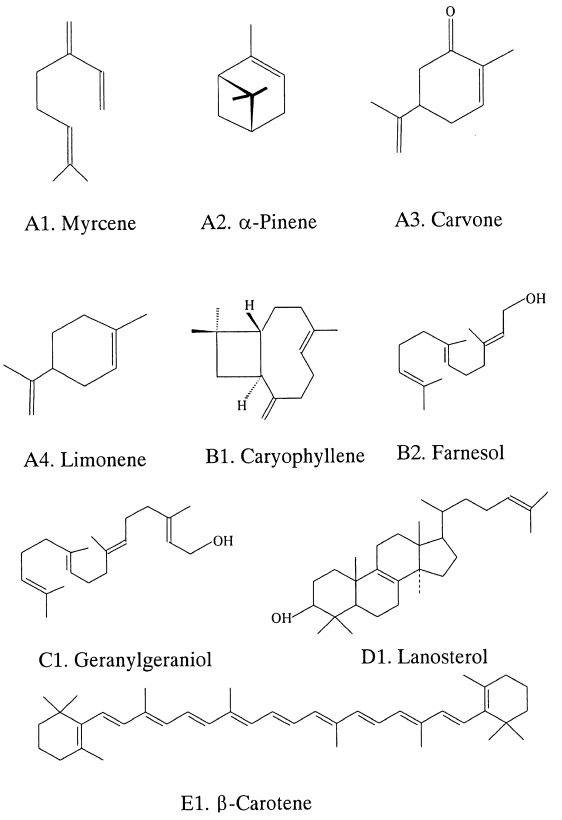

Fig. 1. Structures of selected typical terpenes. (A) Monoterpenes: 1=myrcene, 2=α-pinene, 3=carvone, 4=limonene; (B) sesquiterpenes: 1=caryophyllene, 2=farnesol; (C) diterpene: 1=geranylgeraniol; (D) triterpene: 1=lanosterol; (E) tetraterpene: 1=β-carotene.

whether the performance of the separation will be adequate for the problem. Secondly, once the method has been chosen, the techniques used for characterisation of the separated components needs to be considered. This will largely be the realm of mass spectrometry (MS).

With respect to the first point, the sheer potential complexity of isomeric forms and various chemical classes of components within the broad range of terpenes means that complete separation of a complex mixture may be largely unachievable for a given extract. A compilation such as that of Adams [1] reveals an enormous number of compounds that are present in essential oils and like materials. The similarity of retention indices of many related components shows that component overlap will be the general expectation. The presence of unsaturated bonds, various branched and cyclic compounds, and oxygenated analogues (e.g., alcohols and ketones) will further complicate the issue. Hence essential oil analysts will very quickly embrace new separation technologies to achieve improved analysis. The progression from packed, to capillary, to multidimensional gas chromatography, and then subtleties such as chiral analysis, reflects this need. In most of these developments, there is a desire to increase the ''separation space'' of the analysis [2]. This equates to how many baseline resolved components can be located within the chromatographic run – the so-called separation space. For instance, capillary GC has an operating temperature range the same as packed column GC. Over a given temperature programmed analysis duration, since capillary GC peaks are narrower in time, then more peaks could be baseline resolved. Hence capillary GC quickly supplanted packed GC for essential oil characterisation.

The second point can be introduced simply by stating that the isomeric complexity referred to above will necessarily mean that mass spectrometry will be rather ambiguous for many compounds, giving potential matches with a number of library entries for a chosen component. Thus positive (or confirmation of) identity may rely on other factors in addition to the mass spectrum.

It is against this general background that technologies for essential oil analysis may be explored.

2. Phases

Stationary phase development can generally be seen as a search for specific improved properties conferred by the phase on the chromatographic separation. Thus the developments seek to (i) produce more thermally and chemically stable phases, (ii) give greater selectivity in the separation of components by different phase chemistry (e.g., by developing a different polarity phase), (iii) allow better efficiency by making a more regular surface coating, or producing a thinner film phase coating, or using narrow bore columns, (iv) incorporate specific components to the phase to allow new interactions such as those available with chiral selectors, (v) use different technology to optimise the phase available

to the specific regions of the analysis which require better resolution, e.g., coupled columns of different phases. Almost all of these factors are relevant to essential oil analysis, with perhaps the first being of lesser importance. Since most essential oils elute before the upper temperature range defining high temperature gas chromatography, the use of such thermally stable columns might seem to be less relevant. However, improved stability does also mean that a given column should be more reliable over a greater time period, and this translates to improved long term reproducibility of analysis, which should make analytical characterisation more secure.

Since peak overlap is likely to be of concern, it may be considered advantageous to use a more selective phase for a given target separation. However, there exists the possibility that improved separation of two components using a different stationary phase concomitantly occurs with reduced separation of other components in the sample. If a sample consists of a significant number of incompletely resolved components, then simply shifting them relative to one another without increasing the separation space available within which to fit the peaks offers little net improvement – it is just a reshuffling of peaks to give a different version of an incompletely resolved analysis! This has little overall advantage. One useful point can be made, however, with respect to column polarity. Since a non-polar column will separate (largely) on the basis of boiling point, and since it is quite possible that oxygenated and purely hydrocarbon components might have a similar boiling point, then for a sample that has a wide component composition including oxygenated compounds, the analytical elution range of the sample will be contracted into a narrow retention time range. This contraction of retention time range will lead to greater overlap probability. A more polar column will have the benefit of providing a mechanism for extending the elution range of the components by retaining the more polar components (the oxygenated compounds) to a greater retention time. In this way, the compound classes are pulled apart, and a greater separation space is now available to the sample. Fig. 2 attempts to illustrate this, where some components which eluted in a narrow time range in Fig. 2A are more strongly retained and hence better overall separation occurs in Fig. 2B.

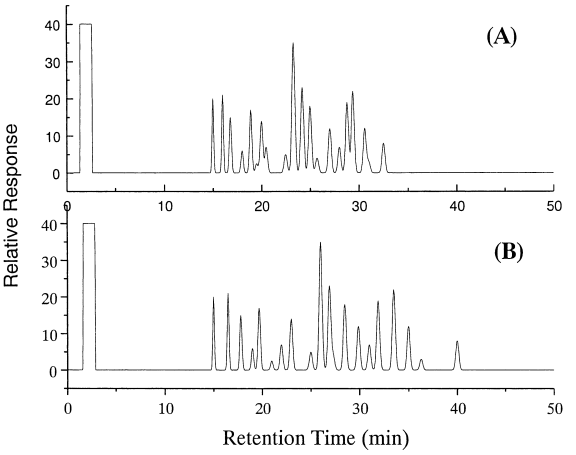

Fig. 2. Illustration of expanded separation space when using a more ''selective'' column. (A) Compounds are restricted to a small elution (temperature) range for example with a non-polar column. (B) By using a polar (selective) column, compounds with polar groups are retained more strongly and so the total sample will be spread over a greater elution range. This leads to better apparent resolution.

An example where stationary phase considerations have been noted is in the compilation of linear retention indices and component amounts for a variety of citrus oils by Dugo et al. [3]. With respect to octanol and α-phellandrene, and limonene and 1,8-cineol separation, SE-52 gave overlaps for these pairs of compounds, and citronellol and nerol, and geraniol and linalyl acetate were difficult to resolve. Carbowax was reported to give better resolution between monoterpene hydrocarbons and their oxygenated products. Many overlaps were noted for monoterpene alcohols and esters with sesquiterpene hydrocarbons. The oxygenated derivatives increase the retention of the monoterpenes to the extent that they elute in the region where the sesquiterpenes are retained. Choosing different phase columns may have little benefit if the net effect is shifting compound classes about to create new coelutions

Increased efficiency from a single column appears to have just about reached a limiting condition for many areas of GC analysis. The use of a 25–50 m long column, with 0.2–0.3 mm I.D. and 0.25 μm phase film thickness will be almost an industry standard. Use of a 0.1 mm I.D. column with a 0.1 μm phase film thickness is much less common. The efficiency increase can be estimated on theoretical grounds, but a narrow I.D. column will generally

require a higher inlet pressure and will generally not be more than 15–20 m long. Hence only a small quantitative improvement may result. The thinner film thickness may result in a column which becomes more active for polar compounds after a shorter usage period, and may give overload (non-linear) conditions for major components. This is detrimental to analysis. Specific column phase chemistry for target analysis such as chiral GC is probably more common now, but still not widely used. Chiral analysis plays a critical role in some areas of essential oil analysis such as authentication of purity of an oil. The natural chiral signature, if well characterised for known natural products, can rarely be reproduced using synthetic compounds, which may simply be unavailable. Thus chiral additives to the stationary phase – the most effective and simple way to achieve chiral analysis – will allow resolution of target components. This theme will be taken up below.

The discussion of the effect of choice of a single column and phase has assumed that the risk of shifting compounds around in a restricted separation space is likely to produce new problems in overlapping compounds. There are technical ways to enjoy the advantages of different stationary phases in an analysis that overcomes this problem, and that involves choosing a particular phase for a particular part of the chromatogram. The only way this can be done practically is to use multidimensional gas chromatography approaches (this method is so important that it must be discussed further; refer to Section 4.2).

2.1. Chiral GC

The development of stable chiral phases for gas chromatography, mostly based upon cyclodextrins, has allowed the enantiomeric compositions of terpenoids and a host of other compounds to be studied in remarkable detail [4,5]. Within the last decades this intellectual curiosity is playing a more important role as a routine analytical and research tool of the essential oil chemist. Such analyses are becoming vital in the industrial setting for the detection of adulterants, characterisation of oil profiles for quality control and when coupled with the development of improved biochemical techniques, has facilitated investigations into the underlying mechanisms associated with the biosynthesis of the terpenoids.

Enantioselective GC in all its different forms has found a wide variety of applications, for instance studies into vitispiranes in grapes [6], epoxygeraniols in coffee flowers [7], Bark Beetle responses [8], piperitone [9], borneol [10], citronellol [11] and α-terpineol [12] in a variety of species and verbenone in rosemary oils [13], and linalool in *Ocimum* species and basil oils [14]. Other work includes fundamental work into the methodology of separating chiral monoterpenes [15], linalool and linalyl acetate in a variety of plant species [16,17], extracts of *Angelica* seeds and roots [18], the composition of rose oxide ketones [19] and other monoterpenoids in geranium oils [20,21], neroli and petitgrain oil [22], rose oils [23], distribution studies of changes during processing of flavours and essential oils [24], tea tree oil and other members of the *Myrtaceae* [25–28], sesquiterpenes [4,29–34] and diterpenes [4,35,36]. Detection of adulterants [16,37,38], the monoterpenes in Scots Pine and Juniper oils [39], *Abies* [40] and *Picea* [41] oils and conifer resins [42], citrus oils [43,44], *Lavandula* species [45], limonene, linalool, citronellal and β-citronellol as authenticity/quality markers in Java-type citronella (*Cymbopogon winterianus*) [46] have also relied on chiral analysis. Biochemical studies into the mechanisms of terpenoid biosynthesis [47,48], the utility of the major monoterpenes in *Larix* species as genetic markers [49] and studies into the biogenesis of the essential oils of *Artemisia annua* [50] reflect other diverse uses of enantiomeric separations. These studies only represent a minor proportion of the studies into the uses where enantioselective GC can and has been employed.

The majority of these studies have used multidimensional GC (see below) to effect the separations; one column separates the peaks as normal and those peaks of interest are cut out of the first dimension and passed onto the second chiral column. However, many studies have used only one column (typically terminated with a mass spectrometer), the commercial chiral columns have separation sufficient for many of the monoterpenoids of interest and can even separate some sesquiterpenes (for instance δ-cadinene can be quite successfully separated on standard SGE or J&W β-cyclodextrin columns) [32,51]. An example of the separation of the primary

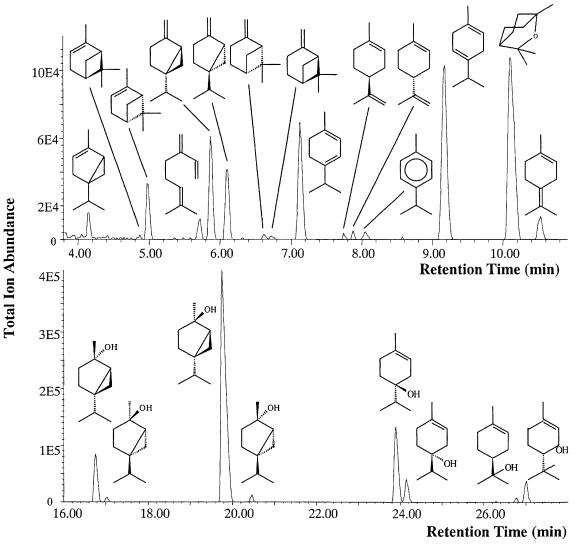

Fig. 3. The monoterpene hydrocarbon and oxygenated monoterpene region (upper and lower traces, respectively) of a chromatogram (determined on a commercial chiral column, J&W CyclodexB, 30 m×0.25 mm I.D., 0.25 μm film thickness) from a typical sample of flush growth from *Melaleuca alternifolia*.

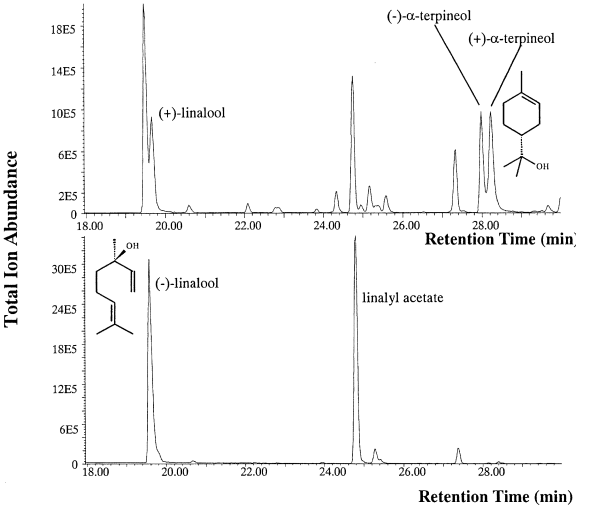

Fig. 4. Chiral chromatograms of Tasmanian lavender oil, showing the simultaneous resolution of (±)-linalool, and (±)-α-terpineol (upper trace). These additional components result from racemisation and rearrangement during acid hydrolysis of the original oil, which is enriched in (−)-linalool (lower trace). The enantiomeric composition of α-terpineol was 56% (+)- and 44% (−)-.

monoterpenes and alcohols (including all four isomers of sabinene hydrate) from the flush growth of *Melaleuca alternifolia* (tea tree) is shown in Fig. 3 [27]. The sabinene and sabinene hydrates are converted into the more familiar α-terpinene, γ-terpinene, terpinolene, terpinen-4-ol pattern associated with tea tree during leaf aging or distillation of the young growth [26,27]. Similar changes can also occur in *Leptospermum* [52]. The partial racemisation of linalool that can occur during distillation is demonstrated in Fig. 4, the lavender oil was heated in the presence of dilute acids at 100°C. The optical isomers of linalyl acetate were not separated on the commercial chiral column used.

Bicchi et al. developed some specialty columns that addressed particular problem chiral separations, noting that certain phases preferentially resolved certain enantiomers [38]. Thus a 30% 2,3-di-*O*-ethyl-6-*O*-*tert*.-butyldimethylsilyl-β-cyclodextrin–PS-086 (polymethylphenylsiloxane) phase allowed characterisation of lavender or citrus oils containing linalyl oxides, linalool, linalyl acetate, borneol, bornyl acetate, α-terpineol, and *cis*- and *trans*-nerolidols. On the other hand, peppermint oil was better analysed by using a phase of 30% 2,3-di-*O*-methyl-6-*O*-*tert*.-butyldimethylsilyl-β-cyclodextrin–PS-086, for the components α- and β-pinene, limonene, menthone, isomenthone, menthol, isomenthol, pulegone, and methyl acetate. The chiral components of rose and iris oils required 50% 2,3-di-*O*-ethyl-6-*O*-*tert*.-butyldimethylsilyl-γ-cyclodextrin–PS-086, which resolved rose oxides, linalool, citronellol, and, *cis*- and *trans*-α-irone, β-irone and *cis*-γ-irone. This points out a problem in chiral analysis. A universal chiral selector that has widespread utility for enantiomer separation is lacking, and thus effective optical separation of all chiral compounds in a sample may be unachievable on any one chiral column.

Direct coupling of a chiral to a non-chiral column has been shown to provide retention characteristics intermediate between those of the two joined phases. Dugo et al. [53] employed a SE-52 primary column and second column of 2,3,6-tri-*O*-methyl-β-cyclodextrin in OV-1701 to separate (±)-limonene and ensure that the (−) enantiomer was separated from an otherwise interfering (unnamed) compound. Hardt and König studied the effect of concentration of cyclodextrin chiral selector and various other oper-

ating variables on enantiomer separation efficiency [54]. An optimum selector concentration of 30% in the OV-1701 achiral carrier phase was found.

3. Aspects of sample handling for extraction and introduction of samples

The range of sample introduction methods for essential oil analysis by using GC is probably not quite as broad as the total technologies available for GC sample introduction. This is largely due to the nature of the studies of general interest in this area. Thus large volume solution injection per se may be of less interest because ultra-trace analysis is not so widely required for these samples. However, methods which permit facile headspace sample injection will be useful, as will interfaced sample separation–injection techniques where the prior separation step achieves some degree of sample clean-up or compound class separation. In this case large volume solution introduction may be the preferred method.

3.1. Extraction methods

Whilst in this brief review only limited comment can be given to extraction methods, some typical applications may be summarised. Firstly, a recent review [55] gave a history and guide to simultaneous distillation–extraction, and presented a range of apparatus by which this process may be conducted. A variety of distillation methods were used at different stages of plant growth and compared the extract component abundance in each case.

Stashenko et al. investigated different extraction methods – steam distillation (SD), distillation–solvent extraction (SDE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), for the isolation of secondary metabolites from *Lepechinia schiedeana* with capillary GC–flame ionization detection (FID) and GC–MS analysis [56]. Heavier components (diterpenoids and phytosterols) were only observed in MAE and SFE extracts. For the mono- and sesquiterpenes and their oxygenated analogues, SDE appeared to give the most favourable recovery. Cherchi et al. [57] extracted *Santolina insularis* essential oil by SFE, studying extraction parameters and comparing the result with hydrodistillation. Some degree of fractionation was observed since lighter and less polar components are removed from the SFE cell faster than higher-b.p. components. Oils extracted by SFE with CO_2 were reported to be less prone to thermal degradation than the alternative SD; SFE also gave some degree of selectivity, and less water interference or hydrolytic processes. Further examples of SFE include: isolation of eucalyptus oils [58], and extraction of semi-oriental tobacco, *Otlja* [59]. The extraction kinetics of SFE was studied for bergamot peels [60]. Again the composition of the extracts changed with time, however two phases of extraction were noted; after a certain period, the extract exhibited very similar compositional properties to that of whole bergamot oil. The component bergaptene's solubility is related to the water content, and so extracts from fresh peels had greater bergaptene abundance. As a final example, pennyroyal oils extracted by SFE were compared with hydrodistillation [61], and quantitatively both isolation methods gave similar content of the monoterpenes pulegone (~80%) and menthone (~9%), but the SFE extract gave a closer olfactory resemblance to the starting vegetable matter.

Recently, there has been a shift in attention towards water as an extractant. Rather than using supercritical water, which can be aggressive, the use of pressurised hot water or subcritical water is now a tested technology. Rovio et al. extracted cloves with pressurised hot water [62], in either liquid or gas (steam) phases. Extracted components were quantitatively recovered on solid-phase extraction cartridges, with fast extraction kinetics (extraction in 15 min) when using the hotter water condition of 250–300°C. Subcritical water was found to give selective extraction of oxygenates from savory and peppermint. The efficiency increased with temperature (for the range studied 100–175°C), and although some components exhibited decomposition the exclusion of oxygen allows preservation of some components that are degraded in hydrodistillation. Results were compared with SFE and hydrodistillation. For some components, 30 min of subcritical water extraction gave a similar quantitative result to 1 h of SFE and 4 h of hydrodistillation. Water gave better extraction of polar components, but less favourable extraction of non-polar compounds. SFE, on the other hand, gave excellent GC evidence of heavy alkanes (hepta-

cosane–tritriacontane), whereas these components were hardly in evidence with the other two water-based procedures.

3.2. Headspace methods

Kolb reviewed the role of headspace sampling with capillary columns [63], describing the process as one of gas extraction which can be carried out as a static or equilibrium process, or as a continuous process (dynamic headspace). Depending on the sample concentration, small gas volumes can be directly injected onto the column, however lower concentrations requiring larger gas volumes with capillary columns necessitates consideration of the injection bandwidth, and thus cryogenic trapping may be used to reduce band spreading and also provide a concentration step. Depending on the temperature of the cooled zone, either cryogenic condensation or cryogenic focusing (where the capillary column stationary phase still operates as a partitioning phase to decelerate the migration of solute) may be employed. In the former case, instantaneous evaporation of the trapped band then permits re-mobilisation of the band. A range of essential oil applications of headspace gas chromatography (HS-GC) were described using various enrichment and cryogenic techniques. Amongst the techniques covered was solid-phase microextraction (SPME). It is clear that the simplicity of SPME has won many converts, and understandably any areas of volatiles/headspace analysis will be an immediate application area for SPME. A selection of chapters in Pawliszyn's book [64] both describe the technique of SPME and various studies on essential oils.

Bicchi et al. described their work on the effect of fibre coating on headspace SPME from aromatic and medicinal plants [65]. As an example, the comparison of static headspace GC (S-HS-GC) with headspace SPME–GC using different polymeric coatings was presented. With rosemary and valerian oils, a polydimethylsiloxane (PDMS) coating gave poor comparison with S-HS-GC, but a more polar coating of CAR–PDMS (Carboxen–PDMS) and CAR–divinylbenzene (DVB)–PDMS produced comparable recoveries of the volatiles. Data were presented that showed most of the other fibres gave reduced abundances of extracted components when normalised against CAR–DVB–PDMS.

Jones and Oldham presented a substantial review [66] of the area of gas chromatography applied to pheromone analysis, and mentioned the use of the contact-SPME method for direct sampling of the components from the sex gland of the female Lepidopteran. It was reported that this method extracted greater quantities of target components in moth sex pheromone studies. Menthol and menthone were determined in food and pharmaceutical products by using SPME, by Ligor and Buszewski [67]. It was reported that the technique was reproducible and gave both qualitative and quantitative analysis at ppm and ppb levels. Calibration curves for menthol over the range of about 25 to 85 mg/l initially prepared in methanol then diluted to final concentration with water gave excellent correlation coefficients.

4. Hyphenated and multidimensional analysis of essential oils

Hyphenation refers to the coupling of spectroscopic detection methods or other specific types of detection techniques, or using different analysis approaches in unrelated (orthogonal) dimensions of an analysis to improve the separation performance or quality of data from an analysis. These can be collectively termed multidimensional methods. Brinkman edited a book devoted to the variety of technologies and approaches underpinning hyphenation [68]. Thus in this category of analysis can be included systems which incorporate separations prior to GC, multi-column separations [multidimensional gas chromatography (MDGC)], and specific identification methods following the GC separation. In this review, only selected examples of each of these approaches will be given as typical solutions that analysts have employed for improved analysis. König et al. [30] briefly reviewed gas phase analytical procedures for sesquiterpenes, and touched on some of the methods reported below such as MDGC, chiral approaches, and isolation methods. Cortes summarised a broad range of multidimensional separation technologies [69], commenting on the increased information content provided by these in-

strumental approaches, and de Geus et al. [70] updated this area by outlining the developments in multidimensional gas chromatography.

4.1. Prior separations preceding GC analysis – HPLC–GC; SFE–GC

HPLC and GC constitute orthogonal separations – they offer different mechanisms of separation, and as a generality HPLC will effect a broad class separation of a sample, and following this the separated fractions may be introduced into a GC system for further high resolution separation based on boiling point and/or polarity. For example, Beens and Tijssen illustrated the value of fractionation of a mineral oil distillate into various chemical class fractions prior to high-resolution (HR) capillary GC analysis [71]. This system was fully automated, but off-line sampling of HPLC fractions may also be introduced in toto by use of large volume injection or volume reduction of the fraction, or may be subsampled to introduce a representative part of the fraction. Large volume injection or complete fraction injection allows conservation of mass of sample analysed, and will be important for low-abundance constituents. Coupled LC–GC in the food analysis area has been reviewed [72]. The HPLC step achieves isolation of components of similar chemical composition, primarily based on polarity, and hence will separate oxygenates, from saturates, from unsaturated/aromatic hydrocarbons. The HPLC technique chosen will determine the particular class separation achieved. Mondello et al. have summarised their approach to this innovation in industry magazines [73,74]. A schematic diagram of such an instrument is displayed in Fig. 5. In this instrumental arrangement, each transferred fraction will be analysed separately before a subsequent fraction can be introduced into the GC system. As an example, three fractions of sweet orange oil (aliphatic aldehydes, sesquiterpene aldehydes and monoterpene aldehydes) were introduced into the GC instrument, and they were compared with the GC analysis of the non-fractionated oil. In another example, use of a chiral GC column allowed the enantiomeric ratios of linalool and terpinen-4-ol to be determined with selective transfer of these components from the HPLC pre-separation step.

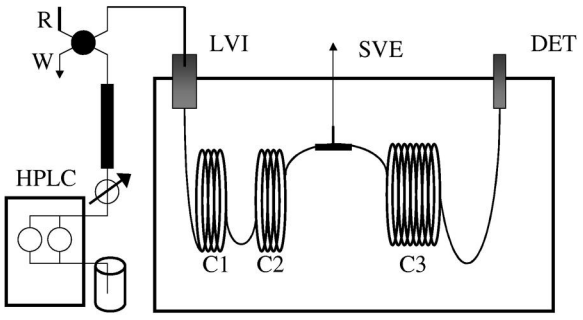

Fig. 5. Schematic diagram of the hyphenated HPLC–GC instrument. The HPLC pump flow passes through the HPLC separation column, and may pass to waste (W) or to the large volume injector (LVI). A restrictor R may be provided at the other port of the four-way valve. On introduction to the GC column, solution passes through a retention gap (C1) and retaining column (C2), with excess solvent removed through the solvent vapour exit (SVE). The analytical column (C3) receives the transferred solute peaks and is detected at the detector (DET).

In a further example, two selected fractions were composed of hydrocarbons and oxygenated components, respectively of bitter orange petitgrain. Normal-phase HPLC with pentane eluted the hydrocarbons, and backflushing with diethyl ether allowed collection of the oxygenates.

The same group have prepared a series of papers – ''*Automated HPLC–HRGC: A Powerful Method for Essential Oil Analysis*'' – in which HPLC–HRGC–MS was used for bergamot [75] and analysis of a wide range of oils [76], and chiral analysis [43]. The authors reported that more accurate results were obtained for the essential oils, due to the better separation and reduced interferences from overlapping peaks, and proposed that this instrumental approach might be a reference procedure for characterisation of citrus essential oils. A review of the area was presented in a further paper by the same group [77] detailing the LC eluent evaporation methods that may be employed to remove the excess of solvent from the injected sample. Selected olive oil samples of different quality were contrasted, and a whole ''nerioli2'' essential oil was compared with its isolated oxygenated and terpenes fractions. Advantages in analysis time in respect of sample separation and cleanup were quoted, with better repeatability and improved quantification.

An on-line SFE–GC system was developed by

Yarita et al. for citrus oils. The silica-gel phase used in SFE elution gave separation based on solute polarity, with three fractions (hydrocarbons, aldehydes and esters, and alcohols) introduced separately into the GC system [78]. Clearly the primary driving force for developing such hyphenated systems is the lack of resolution of the (single column) capillary GC method. The prior separation step will essentially be introduced to simplify the subsequent GC presentation. If the GC analysis could be significantly improved to give much better resolution, then the rationale for hyphenated HPLC–GC or SFE–GC methods may be lost. Methods described below address this aspect.

4.2. Multidimensional gas chromatography

The application of MDGC to essential oil analysis is a logical development in analysis of such complex samples, and was soon adopted as this technology became available. Thus by effecting a heartcut event during a given region of a chromatogram, the desired components are transferred to a second – more selective – column, whereupon the components are better resolved. This case is shown in Fig. 6. Here, two regions of unresolved components are selectively transferred to a second column, where they are now completely resolved. In this diagram, elution order on the second column is altered for the later-eluting region, which demonstrates a different elution mechanism is available to give enhanced separation. In 1975 Schomburg et al. [79] described a double-column chromatography method, where the use of intermediate trapping improved considerably the performance of analysis. A perfume oil sample was analysed isothermally, with retention index values calculated on each column (OV-101 and OS-138; polyphenyl ether polar phase column), and the difference in I values determined. Their novel method involved introduction of an alkane standard from a second injection port into the cryotrapped components to simplify retention index calculation. It appears other studies have not taken up this concept. Many authors have described the virtues of MDGC to increase the separation space of gas chromatography analysis to provide enhanced resolution for given regions of a chromatographic analysis. Schomburg [80] reviewed the technical implementation of MDGC. The typical approach is to isolate a specific region, or regions, of components that elute from one column, and direct these zones or heartcuts to a second column. The usual method will involve cryotrapping at the start of the second column, in order to refocus the transferred band into a compressed region. At some later stage, the cryotrap is allowed to heat up and the components chromatograph on column 2. By choosing a column of different selectivity, the concept requires that overlapping components that were unresolved on the first column now will be better resolved. By taking only small regions of the first column effluent, it ensures that the components do not spread out into sections of chromatographic space that contain other components, illustrated conceptually in Fig. 6. Fig. 7 is a schematic diagram of a typical MDGC arrangement. Major modifications will be to incorporate additional switching valves that might allow choice of different detectors, or use split flows to use parallel detection. Also, some authors have developed dual oven systems where the second column may be independently controlled to analyse heartcuts when they are transferred from the first column. The system in Fig. 7 may require the oven to be cooled to a low temperature prior to turning off the cryotrap and then temperature programming the oven to elute the collected heartcuts on column 2.

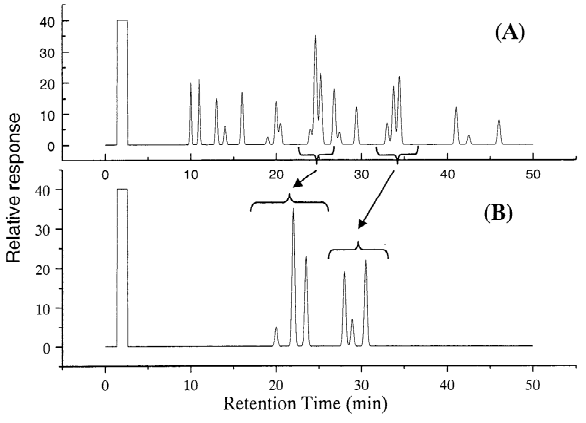

Fig. 6. Multidimensional gas chromatography heartcut concept. Desired regions, which exhibit poor resolution on the first column (A), are transferred to the second column where they are analysed on a more selective phase, which allows their quantitative separation (B).

The role of MDGC is clearly to target a certain

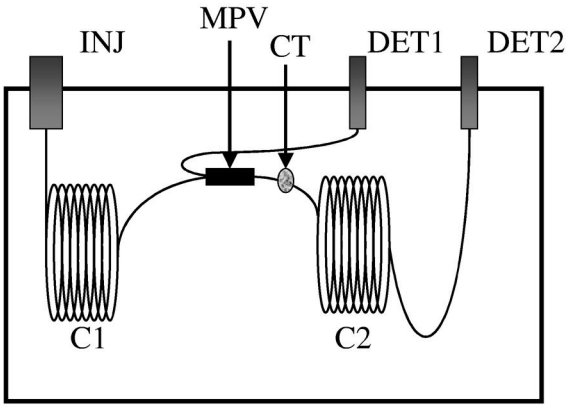

Fig. 7. MDGC instrument schematic. Column 1 (C1) enters the midpoint region and column 2 (C2) is one possible carrier flow exit. When the midpoint valve (MPV) is operated, carrier flows to C2 and heartcut solutes are cryotrapped at CT. The midpoint restrictor connects the midpoint valve to detector 1. INJ: Injector.

poorly separated region and provide increased resolution, and this implies that either quantitation or identification of components is improved through the use of this method. It is important to recognise that the improvement offered by MDGC is only available to a relatively few regions of a chromatographic analysis because it is just as important to exclude from the heartcut zones those solutes that would cause potential overlap problems on the second column and which were adequately separated on the first column. Using conventional MDGC technology, it is not possible to apply the MDGC advantage to the whole analysis using the heartcut approach, since this would involve transferring all the components to the second column – and this is equivalent to just analysing the sample on column 2. Giddings recognised [81] that the power of MDGC really lay in precisely achieving this outcome, however it required a technical solution that was at that time unavailable in GC. This will be explored later. Bertsch [82] provided a comprehensive survey of the state-of-the-art of MDGC recently, and this is an excellent overview for any reader interested in the evolution, theory, techniques, and applications of MDGC. A variety of oil analyses were included.

Often the target analysis in MDGC of essential oils is not directed to increased resolution of the majority of the sample, but towards specific components whose relative abundances may be required in order to study a particular aspect of the sample quality, history, source or biogenesis. Thus MDGC provides the necessary separation to answer questions which cannot be addressed by single column analysis. A major application area will be chiral analysis; the typical approach will be to use a conventional column in the first dimension, and a chiral column as D2. When correctly employed, a single (unresolved) peak can be transferred and column 2 provides baseline resolution of the enantiomers, without any interfering peak overlaps. A single chiral column will always lead to some uncertainty when trying to resolve chiral compounds in a complex mixture, because there might be interference with either of the enantiomers from unresolved interfering peaks. Even if a mass-selective detector is available, independent identification of overlapping components relies upon the availability (and correct choice) of unique ions for each. Bernreuther et al. used an SE-54 precolumn and Lipodex B (alkylated α-cyclodextrin) chiral column to separate chiral forms of γ-lactones, from different fruit extracts [83]. The *R*-enantiomer eluted prior to the *S*-enantiomer. A subsequent paper by the same group [84] studied the enantiomeric ratio of massoilactone (which has a coconut-like aroma) from a variety of natural sources, and found the compound to be the optically pure *R*-isomer in these samples. The same column combination as before was used. Fuchs et al. briefly summarised their research into biogenesis of essential oils [85], employing SPME with MDGC–MS for chiral analysis. Labelled (^{2}H and ^{18}O) precursors of pulegone were administered to the plant, and the MS detector is able to differentiate the genuine and isotopomer monoterpenes which contain labelled atoms.

In a study of essential oils from Madagascar, Mollenbeck et al. used GC, GC–MS and MDGC to provide the desired separation, including chiral separation, of a large number of components from 12 samples. Enantiomeric excess of five compounds – limonene, linalol, terpinen-4-ol, α-terpineol and citronellol – were reported [86]. The column set of choice for chiral analysis was a first column of DB-Wax coupled with a 2,6-dimethyl-3-*O*-pentyl-β-cyclodextrin–OV-1701 coated second column for the enantiomers of the first four components listed above, and a 2,3-diacetyl-6-*tert*.-butyldimethylsilyl-

β-cyclodextrin–OV-1701 coated second column for the enantiomers of citronellol.

Mondello et al. have applied multidimensional gas chromatography techniques in a series of papers on the analysis of various oils. The first paper illustrated development of an automated tandem GC–tandem oven system [87]. The application again was chiral GC, with a first column of SE-52 and second of diethyl-*tert*.-butylsilyl-β-cyclodextrin phase. Lemon oil, with enantiomeric ratios and relative amounts of sabinene, β-pinene, limonene, linalool, terpinen-4-ol and α-terpineol was presented. Selected subsequent papers in this series presented enantiomeric distribution of monoterpene hydrocarbons and alcohols in mandarin oils [88], and in lemon oils [89], and monoterpene hydrocarbons, alcohols and linalyl acetate in bergamot oils [90].

Fig. 8 is a typical example of MDGC applied to study of an essential oil (cold-pressed mandarin oil) for enantiomeric compound analysis. The top trace is the whole oil with peaks of interest listed. The next analysis will involve setting up the heartcut event times to transfer the desired regions to the second column. Note that operation of the heartcut valve is accompanied by a spike on the GC trace. Once the heartcuts have been collected in a cryotrapping zone they can be eluted by a subsequent temperature program, and by using a chiral column the required enantiomers are separated.

4.3. Selective and spectroscopic detection

4.3.1. Olfactometry

Gas chromatography–olfactometry detection (GC–O) may be considered a biological sensor for identification of the separated components from a GC column. Olfactometry adaptors (sniffer ports) are available from manufacturers such as Gerstel (Germany) and SGE International (Australia), and should include the ability to humidify the GC effluent at the nose adaptor and provide auxiliary gas flow. The correlation of eluted peaks with specific odours allows accurate retention indices or retention times to be established for the components, and the GC–FID results can indicate the relative abundance of the components. Interestingly, it may be observed that distinctive odours arise where no apparent peak is found on the normal GC trace.

Nishimura used an off-line multidimensional gas chromatography system, comprising trapping of selected effluent bands on a porapak tube, for transfer to a thermal desorption/cryotrap unit on the second GC instrument, for analysis of the oxygenated hydrocarbon fraction of ginger odorants. The odour of enantiomers of ginger was a target of the study [91]. The effect of selected operating factors on efficiency of the sniffing port has been studied [92].

A chemical sensor array, comprising a 32-sensor device, without prior GC separation, was used in a method termed olfactroscopy [93]. Chemical differentiation of the samples of *Hypericum* L. species must be done by chemometric approaches, and principal component analysis of the results from the sensors. It was advised that this approach is of advantage for classification of plant species prior to use of ''finer'' analytical methods such as GC and GC–MS. The actual value of the tool, especially if GC–MS is still recommended, was not clearly delineated. This ''electronic nose'' was able to classify perfumes into their perfume families [94]. A total of 18 semiconductor sensors were used, with humidified atmosphere. The headspace injection volume was found to be the most important variable in this experiment, which is not a sample compositional variable.

4.3.2. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry has probably done as much for chromatography as chromatography as done for mass spectrometry. The essential feature of mass spectrometry for essential oils is that mass spectra are not particularly unique in many cases. Thus within the broad class of monoterpenes, a large number of isomers of the same molecular formula but with different structure exist; their mass spectra may also bear close resemblance. However, MS does still give very valuable data for routine analysis. Once one has established a familiarity with component elution time and combines this with MS, the quality of data increases commensurately. A technical solution based on spectroscopic detection (infrared and mass spectrometry) in combination with multidimensional gas chromatography was developed by Wilkins [95]. Using a series of parallel cryotraps, the instrument can be instructed to collect desired segments of the effluent from the first

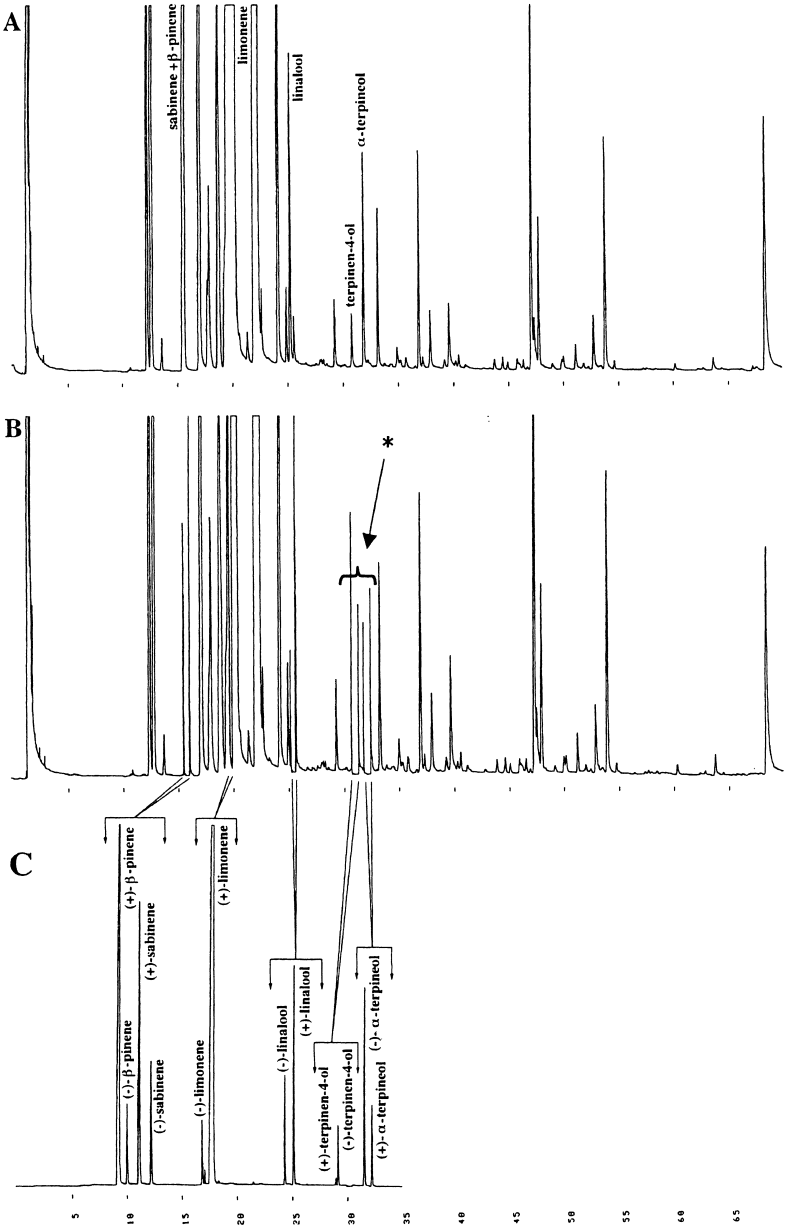

Fig. 8. MDGC analysis of cold-pressed mandarin essential oil sample. A SE-52 first dimension column gave the result in (A). Heartcuts were implemented as shown in (B) to isolate the named compounds from (A), and pass them to the chiral second column. Note that there is an electronic signal indicating where the heartcut operations were conducted, as given by the asterisked parentheses for terpinen-4-ol and α-terpineol in (B). The chiral column analysis in (C). allowed quantitative separation of each of the enantiomeric pairs of components. (From Ref. [88], with permission).

column (precolumn) by diverting the carrier flow to the cryotraps. The system was demonstrated for eucalyptus oil analysis [96]. Using single-stage GC (i.e., a single column), it was shown that a peak in both authentic and adulterated oil, which had the same retention time, had different spectra. Two-stage

(dual column) analysis allowed analysis of the selected peak, and it was shown to comprise a number of components (camphor and 4-terpineol).

Although GC–MS is a basic tool of many laboratories, and literature reports incorporate results obtained from such an instrument as a matter of course, this review will only highlight one aspect of GC–MS for enhanced analytical capability, where compound retention and mass spectral correlation is employed (see below). However one pertinent point requires mention. In the absence of mass spectral data, a non-spectroscopic detector (e.g., flame ionisation detector) can only provide a net response for whatever elutes from the column. Since complex oils may have multiple overlapping peaks, then there will always be uncertainty as to the purity of any recorded peak. If one cannot be assured as to the purity of a peak, then quantitation of a component based purely on FID data may be erroneous. Mass spectrometry may give the ability to recognise overlapping peaks and apportion relative amounts of components where overlaps occur. However, ideally greater (complete) component resolution should still give more reliable quantitation.

Accurate mass (high-resolution) MS is a valuable tool for confirmation of the molecular formula of a detected component. With reference to essential oils, and in particular terpenes, the main problem is that of recognition of the retention time difference that different structural isomers have. For instance within a group of saturated monoterpenes, the molecular ion will have the same nominal and accurate mass. Amongst the class of molecules which have one oxygen atom, again the accurate mass analysis will be of little additional value over low-resolution mass spectrometry. There is sufficient difference in the type of spectrum for saturated and oxygenated components to not need to rely on accurate mass methods. Claude and Tabacchi [97] used a triple quadrupole mass spectrometer in conjunction with MDGC to analyse complex mixtures. High-resolution GC could not resolve all peaks, so acquiring high quality MS data is difficult. A highly versatile MDGC, allowing a range of different operation processes, and collisional activation MS produced superior results.

Specific application areas dependent on mass spectral detection are those involving isotopic labelling of natural products with stable isotopes, especially for biogenetic studies, as mentioned earlier. Thus feeding labelled precursors to a plant allows study of the biochemical incorporation of the label into products derived from that precursor.

One of the newer methods proposed to give improved analysis of complex mixtures, especially for deconvolution of overlapping mass spectra, is time-of-flight mass spectrometry – GC–TOF-MS. Being capable of generating instantaneous spectra, there is no bias arising from the mismatch between scan rate (duty cycle) and peak abundance changes in the ion source which may arise with quadrupole mass spectrometers when used for fast GC peaks, and so one should expect uniform mass spectra across the whole peak. This allows routines (proprietary) to permit assignment of spectra to each individual solute in significantly overlapping elution profiles. This is now promoted as permitting fast GC methods for complex samples since peak overlaps may be deconvoluted and the individual spectra of each overlapping solute obtained. There have been some applications presented in the literature for essential oils by using GC–TOF-MS [98,99], but full evaluation of the method is still awaited to determine if it can offer on a routine basis the performance that is claimed. The fast spectral acquisition capabilities (100+ mass spectra/s) is certainly compatible with fast GC techniques which are attracting much attention recently, and also is a suitable technology for GC×GC (see below).

4.3.3. Retention index – mass spectral correlation

Either retention time or mass spectral information alone is insufficient to provide positive identification of many essential oils. The similarity of mass spectra for a series of terpenes of the same nominal empirical formula makes automated identification based on this data problematic. This is particularly the case for the sesquiterpene hydrocarbons, of which approximately 400 are known to date [30], and many of these yield identical, or almost identical mass spectra. The GC–MS system provides an orthogonal analysis result and so should provide additional data beyond that available from either ‘‘dimension’’ alone. The value of mass spectrometry is usually related to its identification power, over non-spectroscopic detection methods used in GC. This implies

that conventional detectors such as flame ionisation detection (whilst having a useful and well characterised response relationship), are lacking when the retention time property of a component is not enough to reliably confirm the identity of the compound. This again is a result of the possible complexity of the essential oil samples, and the relatively limited range of molecular structural types, which causes clusters of many compounds within a restricted retention range within the chromatogram.

A superior approach is to combine the specificity and/or reproducibility of retention time of components with the mass spectrometry pattern of suspected components with that given retention time. This provides two independent parameters on which to base identity of the compound. Compilations such as that of Adams [1], and Jennings and Shibamoto [100], list the retention index of compounds and their mass spectra in order of elution. Davies [101] compiled the work of several authors, providing a comprehensive summary of retention indices on dimethyl polysiloxane and Carbowax 20M stationary phases. The use of retention indices in conjunction with GC–MS studies is well established and many analysts use such procedures in their routine analysis to confirm the identity of unknown components [102–106]. Of course, there are certain assumptions related to the experimental conditions under which the reference data were obtained. The Adams retention data were acquired on a DB-5MS column (5% phenyl–polydimethylsiloxane phase, 30 m× 0.26 mm I.D., temperature programmed from 60 to 240°C at 3°C/min). Jennings and Shibamoto used a temperature program of 2°C/min, with an initial oven temperature of 70°C (polyethylene glycol Carbowax 20M 80 m×0.2 mm I.D., programmed to 170°C) or 80°C (methylsilicone OV-101 with 1% Carbowax 20M as antitailing additive, 50 m×0.28 mm I.D., programmed to 200°C). Temperature appears to have a relatively small effect on the retention indices of terpenes on dimethyl polysiloxane stationary phases, but can have marked effects on the indices on Carbowax 20M [101]. Use of a column with a different phase coating, of different dimensions, or from a different manufacturer will lead to uncertainty in retention comparison viability. Furthermore, an ion trap mass spectrometer may also give different MS spectra to a quadrupole, and lead to different MS quality matches for spectral comparison [107]. A specialist essential oil laboratory may be advised to develop their own compilation of reference data under their own standard conditions. Mondello and co-workers have reported an automated search routine which essentially couples a private MS library with retention time to provide a better identity of components [108,109]. The availability of MS instrumentation means that the alternative method of employing two parallel capillary columns of different phases for characterisation of samples, whilst still of value in many studies, probably is no longer considered as powerful a tool for positive identification as methods based on mass spectrometry.

Notwithstanding the wide reporting and use of linear retention indices, there must be a note of caution when using such indices in an absolute sense. Data from one laboratory to another will invariably be inexactly reproduced, however the importance is that combined with mass spectral results, retention data does still provide an excellent guide to possible identities of components.

5. Comprehensive two-dimensional gas chromatography

The recently described technique of comprehensive two-dimensional gas chromatography (GC×GC) addresses a number of shortcomings of conventional multidimensional gas chromatography when analysing very complex samples, or samples where the occurrence of overlapping peaks of different polarity arises. It essentially is the technical implementation of the Giddings concept referred to earlier [81]. As early as 1987, Giddings stated [110] that two-dimensional continuous (and coupled column) separation had not at that stage been developed to reach its potential in chromatography. GC×GC is a true MDGC method since it combines two directly coupled columns, which provide orthogonal separation of compounds on the two columns, and importantly is able to subject the total sample to simultaneous two-column separation. Hence the need to select heartcuts, as used in normal MDGC, is no longer required. Since components now have retention in two separate columns, and since the

second column brings its own separation power to the analysis, the net capacity (i.e., total number of resolvable peaks) is the product of the capacities of the two participating columns. As an example, if the first column, which is usually of conventional dimensions, has a capacity of 400 peaks, and if the second (which is normally a short column, with a relatively low capacity) has a capacity of maybe 15, then the system should have a capacity of $400 \times 15 = 6000$ separable peaks if the total separation space was useable. This certainly expands the separation space, and even on a statistical basis should lead to a considerable increase in resolvable components. A single column cannot achieve anywhere near this capacity, and so must be considered of limited separation performance. It has been proposed that for a single column to give equivalent capacity, it may have to be 100 km long.

5.1. Introduction to comprehensive two-dimensional gas chromatography

The GC×GC experiment consists of directly coupled columns, with a modulation device between the two columns, whose function is to zone compress small regions of the effluent from the first column, and rapidly inject or introduce it to the start of the second column. If the first column is non-polar, leading to separation based on boiling point, then it may be assumed that compounds that co-elute on the first column can have quite different chemical property, which can be used as the basis for separation on the second column by ensuring that the separation mechanism of the phase is able to distinguish between these compounds. Fortunately, it may be as simple as choosing a polar column in order to resolve such compounds, or maybe a column which selectively retains aromatic compounds compared with aliphatics. Thus a combination of non-polar (e.g., 5% phenyl–dimethylsiloxane phase) column with a polar polyethylene glycol phase column may be a good first choice for a suitable dual column set for essential oils. Fig. 9 presents a schematic diagram of a GC×GC arrangement in a GC instrument. Note the apparent simplicity of the instrumental arrangement with respect to the column coupling, with no valving or switching systems required.

The modulator is the key to the performance of the

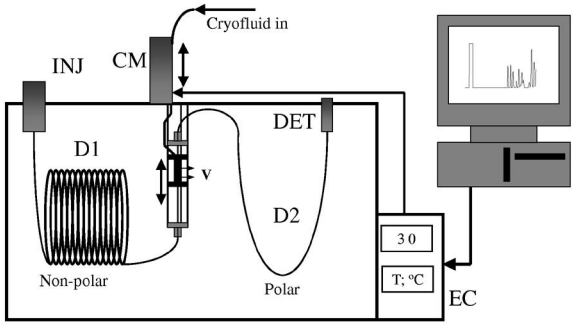

Fig. 9. Schematic diagram of the GC×GC arrangement utilising a cryogenic modulator. CM: Cryomodulator; INJ: injector; DET: detector; D1, D2: separation dimensions 1 and 2; EC: electronic controller with selectable modulation duration (30=3.0 s) and cryotrap temperature shown; V: exit vents from the cryotrap. The modulator oscillates as shown by the arrow. Cryofluid CO_2 is provided to the trap via the movement arm.

GC×GC experiment. Phillips and Beens reviewed a number of approaches to achieving this goal [111]. Bertsch has recently reviewed the field of multi-dimensional gas chromatography and completed the two-part study with a status of GC×GC [82,112]. The first practical modulator technology was the thermal sweeper method described by Phillips and Ledford in 1996 [113]. Based on a development dating from 1994, the present senior author described a cryogenic modulating system [114,115], that could also be effectively used for GC×GC [116,117]. The two systems were compared in a collaborative study, and shown to give equivalent results [118]. Other arrangements for achieving a continuous two-dimensional separation based on valve operation have been described, such as that of Bruckner et al. [119].

The key to the GC×GC technique may be listed as follows.

(i) Setup an arrangement of two directly coupled columns with a modulation system located at or near their junction. Column 1 is of normal dimensions, and column 2 is a short column.

(ii) Modulators that operate under mass conservation collect contiguous zones of effluent from the first column, zone compress them in space and time and deliver these packets of solute as very sharp pulses to column 2.

(iii) Column 2 operates as a fast GC column and allows analysis of these zones in a very short time period. It is useful if the time duration of analysis on

column 2 is less than that of the time of the pulsing process, but this is not a strict requirement.

(iv) Each peak eluting from column 1 should be sliced into several segments by the modulation process. This means that for each solute there will be as many separate analysed peaks on column 2 as there are modulation events during that compounds' elution. Thus if a solute is sliced into five separate zones, there will be five pulsed peaks produced by column 2 and recorded by the data system. Each of these peaks will essentially be separated in time by the modulation period used.

(v) Since column 1 may have unresolved compounds that are collected together and zone compressed in the modulator, the ability to separate these on column 2 will lead to a much greater separation of compounds in GC×GC. The peak capacity is greatly increased and so achieves Giddings' vision of the ultimate two-dimensional experiment. This requires that the separation mechanisms of the two columns should be different, and so an orthogonal analysis is achieved.

(vi) Data are converted into two-dimensional plots to allow a two-dimensional presentation of the chromatogram. Thus each peak will be an oval-type shape when presented as a contour, or a three-dimensional volume when retention height is also shown.

(vii) The pulsing and fast analysis process leads to significant peak response increase, and so greater sensitivity of analysis is obtained.

5.2. Comprehensive gas chromatography for essential oil analysis

GC×GC has been largely described for petrochemical and related samples [120,121]. This is the historical situation because such samples were (i) known to be highly complex, and so ideally suited to demonstration of a high resolution separation, and (ii) were of interest to the Shell company, whose involvement in developing this technology is acknowledged, and which lead to the petroleum predominance. The application to petroleum biomarker identification has extended the GC×GC method in that area [122]. However, more recently a broader range of analysis applications have appeared, including sterols [123], organochlorine pesticides [124], semivolatile aromatics [125] and atmospheric organics [126].

However, the complexity of essential oils is ideally suited to two-dimensional analysis, which is both why MDGC is applied in this area, and also why GC×GC should be a fertile analysis area. There are only limited reports of GC×GC of essential oils [127–130], at the present time, primarily because of the few groups who are working in this area, but results thus far foretell of a very promising future. A brief summary of the studies, and where they may hold significant relevance to this area will be given below.

Essential oils are largely composed of a range of saturated or partly unsaturated cyclic and linear molecules of relatively low molecular mass, and within this range a variety of hydrocarbons and oxygenated compounds (e.g., hydroxy and carbonyl derivatives) occurs. There is an obvious opportunity to exploit the likely polarity differences of closely eluting compounds on the first column by choice of a suitable phase on the second column. Provided there is a separation mechanism which permits their resolution on the second column, then the coeluting components from the first column will subsequently be resolvable on the second column. This is not too unsurprising for overlapping compounds which represent different classes of compounds (e.g., saturated hydrocarbon and a hydroxy-substituted compound), but the ability of the second column to resolve different saturated hydrocarbons will be a more stringent test of the method. The most striking potential of GC×GC was demonstrated in a study of vetiver oil (*Vetiveria zizanioides*) [128] in our laboratory. Vetiver from Haitian sources has been studied in some detail recently by Weyerstahl et al. [131], with 144 components reported in the neutral fraction of the oil along with their relative retention indices which indicated considerable overlap of components; its complexity has been acknowledged for some time [132]. The GC×GC result as given in the two-dimensional plot in reference [128] proposed that series of compounds might be identifiable amongst the compounds that elute prior to the largest peak in the sample, with these series occupying a relatively narrow retention band on the second dimension column suggesting that they have similar retention parameter (and also retention time) due to their

similar structures (e.g., they may be largely hydrocarbon-type compounds). After this large abundant component, it is believed oxygenated compounds contribute to a greater degree of spread in the second dimension retention, and so greater retention differences and less apparent retention relationships. Fig. 10 presents a typical GC–MS analysis of this sample. However, attempted correlation of peaks with proposed identities using Adams' [1] or Weyerstahl et al.'s [131] compilations still does not allow certainty in assignment of identity. It is apparent that increased resolution will be of much advantage in this sample. Fig. 11 demonstrates how the chromatographic results from vetiver oil may be viewed in a sequential development mode, with initial non-modulated presentation (Fig. 11A), through the modulated experiment showing the pulsed peak responses of solutes (Fig. 11B), and finally the two-dimensional separation space (Fig. 11C). Enhanced sensitivity of the modulated chromatogram is indicated by the response of the pulsed chromatogram being some 20+ times greater than that of the normal GC trace. The Fig. 11B inset displays an expanded region that shows the series of pulsed peaks arising from the modulation process. Thus at least five compounds (indicated by letters a–e) coeluted on the first column at a retention time of about 56 min. A slow temperature program was used (1°C/min) and so broad peaks entered the modulator and gave up to six pulses at a modulation time of 4 s.

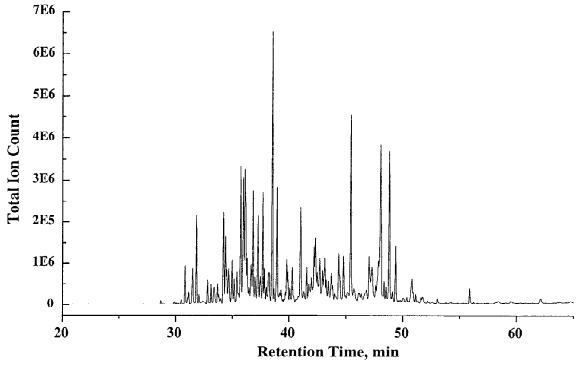

Fig. 10. Typical analysis of *Vetiveria zizanioides* (vetiver) oil by using GC–MS analysis. Agilent 6890GC with a 5730 mass-selective detector, and a BPX5 capillary column (SGE International). Temperature program of 45°C, held for 6 min, then temperature programmed at 3°C/min to 250°C. Initial column head pressure 3.47 p.s.i. (constant flow mode; 1 p.s.i.=6894.76 Pa).

The two-dimensional result (Fig. 11C) clearly illustrates that there are considerable overlaps of components on the first column, and so this implies that the conventional analysis of such an oil will be unlikely to even hint at the complexity of the sample. For instance, GC–MS would have to be able to uniquely identify as many as 8–10 coeluting components. Of major importance is that since components are baseline resolved, the low or trace abundant components can be readily recognised even when they coelute with major components on the first column. At a retention time of 56.15 min (given by the vertical line on Fig. 11C), it can be seen that five peaks will be overlapping on the primary column. The contour plot of minor peaks, e.g., as identified by (a) or (b) in Fig. 11C, can be clearly and unambiguously found and so now it is readily apparent how many solutes overlap at any point on the first column, and importantly now they can be measured. In a study of peppermint and spearmint, Dimandja et al. [127] were able to match certain components in common in the two oils simply by alignment of their two dimension retentions. Likewise, Shellie et al. showed that authentic standards of compounds found in lavender [129] could be matched with their respective peaks in the analysed lavender oil sample. The use of fast detection time-of-flight mass spectrometry for GC×GC of lavender [130] illustrated the approach that can be taken for MS detection in GC×GC, with interleaved peak pulses where primary column overlapping compounds were resolved on the second column. Each of the peak pulses was separately identified by library comparison, and the result is given in Table 2. Each peak pulse is separated by the modulation duration (4 s). Fig. 12 is a schematic representation of how two overlapping peaks on the first dimension column are pulsed into a series of interleaved peaks, (A) and (B), in the second dimension, which are now completely resolved. Since all of the (A) peaks are the same compound, they will all have the same mass spectrum, so here five pulsed peaks will be identified by library matching as being the same compound.

5.3. The future of GC×GC for essential oil analysis

Where might the technique of GC×GC be di-

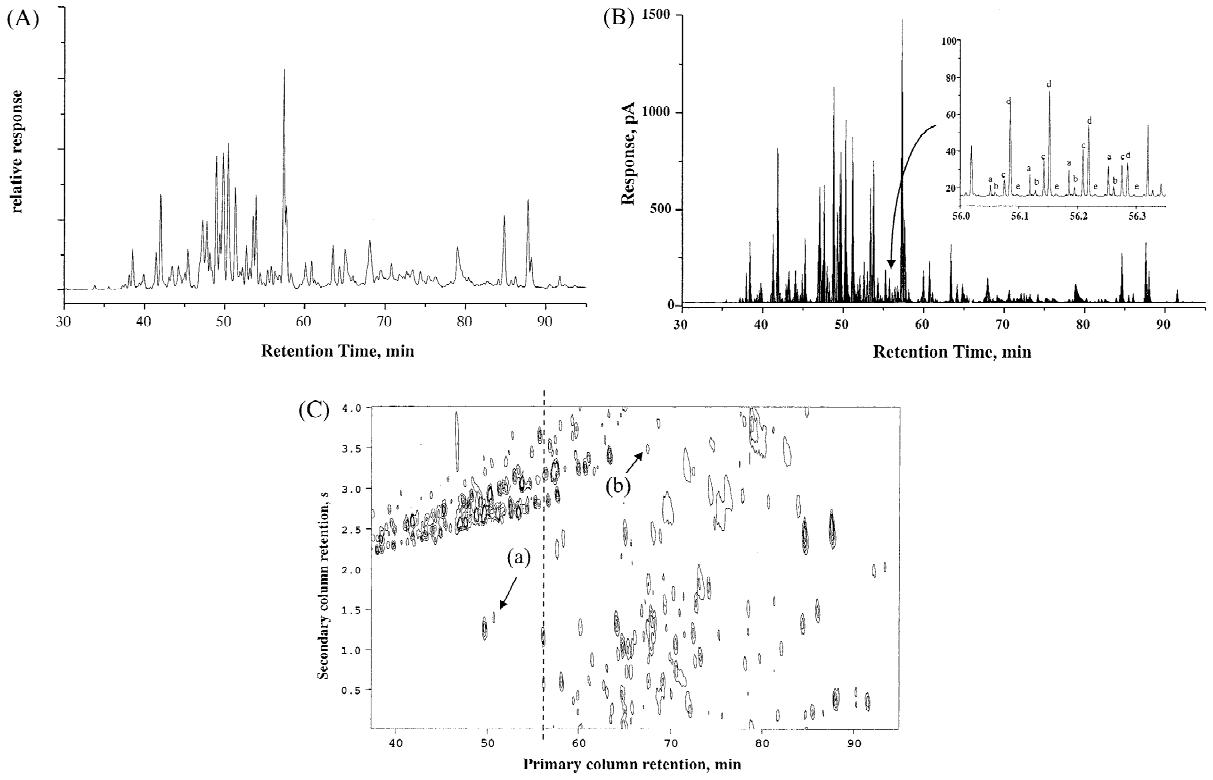

Fig. 11. Vetiver oil GC analysis using a column set of BPX5–BP20 (20 m×0.25 mm I.D., 0.25 μm film thickness, and 2 m×0.1 mm I.D., 0.1 μm d_f, respectively). Temperature program of 60°C, held for 1 min, then temperature programmed at 10°C/min to 120°C, and temperature programmed at 1°C/min to 240°C. Initial column head pressure 4.74 p.s.i. (constant flow mode). Modulation period was 4.0 s, with 0.5 s release time. (A) In this case the GC×GC arrangement is used, but without cryofluid supplied to the trap, hence a normal GC result is obtained. (B) Same sample and conditions as (A) but modulated cryotrapping is used to obtain GC×GC results. This trace is a presentation of the pulsed chromatogram. Modulation duration is 4 s. (C) Data from (B) are transformed into matrix using the modulation duration to generate a two-dimensional chromatogram space. Each contour peak is a separate compound.

Table 2
Retention times and identities of peak pulses found in GC-TOF-MS of lavender oil where borneol and terpinen-4-ol are unresolved on the primary column [130]

Retention time of peak pulse (s)	Peak (A) identity from automated library search	Peak (B) identity from automated library search
1273.5	Borneol	
1277.5	Borneol	
1279.1		Terpinen-4-ol
1281.2	Borneol	
1282.8		Terpinen-4-ol
1285.2	Borneol	
1286.8		Terpinen-4-ol
1290.9		Terpinen-4-ol

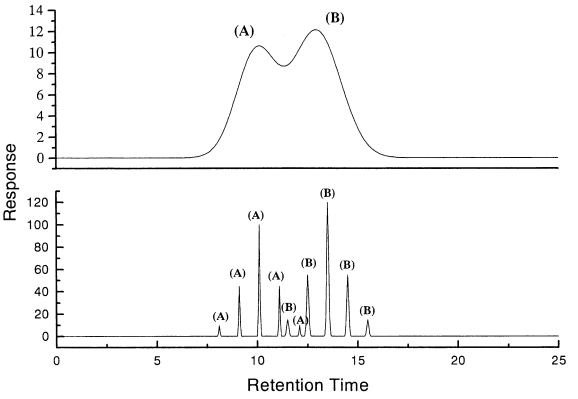

Fig. 12. Conceptual representation of resolution of two peaks which are unresolved on the first column (top) but by the pulsing process and orthogonal analysis conditions are resolved on the second column (bottom). When using a mass spectrometer, peaks labelled (A) will all be identified by library searching as the one component, and those labelled (B) will be the other component.

rected in the future, and what can be expected of developments in this area? Firstly, there is an active research effort in demonstrating the application of the technique to a wide diversity of sample types – clearly there are many years of conventional capillary GC experience to draw upon, and also many examples in the literature where inadequate separations are illustrated. Thus an expanded applications data base will soon result. There are many considerations that must be taken account of when implementing GC×GC methods, and these will only be routine and/or logical when there have been sufficient studies completed that place the operation and optimisation of the GC×GC approach into context and allow rational choice of operating conditions. For instance, a faster temperature program ramp rate delivers solutes to the second column at a higher temperature, and therefore leads to shorter retention on that column. This could affect second column presentation and separation. Thus there will be an expanded effort to better define the fundamental (interactive) parameters of the GC×GC experiment. All of these studies will bring to essential oil analysis the improved understanding of system operation and technique implementation developed across all areas of applications. Essential oils will pose their own challenges, and in particular the role of chiral analysis will soon be studied in detail by using GC×GC, with the potential for significantly simplifying the approach to chiral analysis. The choice of column sets will probably be limited to a small number of recommended dual columns, since this will allow advanced pattern recognition or retention correlations to be used to compare and contrast a variety of samples. This is similar to the retention time (index)/mass spectral correlations in single column studies of essential oils. One of the major expectations of analysts in this area must be the availability of mass spectral data for separated components, and similarly to validate that the incredible range of separated peaks are meaningful compounds, GC×GC–TOF-MS will be a further important technology by providing MS data. Presently TOF-MS is the only viable technology for fast mass spectral data acquisition for peaks that may have basewidths of as little as 100 ms. It can be predicted that major efforts in this area will soon be reported. Whatever the future holds, it can be reasonably assured that GC×GC will hold many surprises and much value in respect of new information derived from GC analysis.

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