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Multiple component isolation in preparative multidimensional gas chromatography with characterisation by mass spectrometry and nuclear magnetic resonance spectroscopy

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ABSTRACT

The preparative scale isolation of multiple components from an essential oil matrix is described using multidimensional gas chromatography (prep-MDGC) which allows their further characterisation by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. Menthol, linalyl acetate, carvone and geraniol were isolated individually, and were also collected in various combinations. It was demonstrated to be possible to collect multiple selected components from numerous repeat injections of the sample, to permit increased mass recovery from an external cryotrap collection device. Peak retention times remained reproducible (<0.3 s) over the repeated injections and switching events. This methodology may be utilised to confirm peak identity or to produce unique mixed-component reference standards, for instance to allow their identification in other samples using GC/MS, or identify them in comprehensive two-dimensional gas chromatography (GC × GC) analysis.

1. Introduction

Multidimensional gas chromatography (MDGC) is attracting resurging interest from the GC community, as improved instrumentation and procedures have become more widely available. This is reflected by an increasing number of research and application studies as indicated below. The history of classical MDGC, which today includes pneumatic flow-switching (Deans switching; DS [1]) and other various valve-based heart-cutting devices, commenced relatively soon after the introduction of single-column gas chromatography [2]. Books dedicated to multidimensional chromatography [3–5], and reviews by Schomburg [6], David and Sandra [7] and the more recent overview of Bertsch [8], testify to both the ingenuity of chromatographers seeking new ways to augment the basic GC methods, and that the needs for enhanced separation methods reveal that the required peak capacity is unsatisfied by the basic single-column GC method.

Whilst there have been a number of devices that offer different mechanisms to the heart-cut process, such as the Live-T switching device (Siemens Sichromat) [6,9,10], the Gerstel column switching system [11,12] and the moving capillary stream switching (MCSS) device [13], it is largely the field of micro-fluidics that has given a new lease of life to MDGC [14,15]. MDGC has become a more 'user-friendly' technique due to improved column connections, superior pressure/flow control, and the support of computer software to aid calculation of column dimensions and experimental conditions.

In selected prior research, Wilkins and co-workers [16–18] reported spectroscopic detection capabilities with GC, and in particular with MDGC. The use of a valve-based interface between 1st and 2nd dimensions was reported to have no deleterious effect upon the components studied. Krock et al. [19] studied cryotrapping of narrow (12 s) and broad (72 s) cuts from the first dimension,

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noting that in the former, improved performance of the analytical column resulted. The work of Gordon et al. [20] is also noteworthy for the sheer complexity that was demonstrated in a flue-cured tobacco essential oil. Using multiple heart-cuts, a long ^{2}D column and long analysis times, the authors identified a total of 308 compounds, with 80 found as tobacco constituents for the first time. This indicates the capability for discovery of new compounds through application of MDGC approaches.

The present group has recently been involved in the development of new capabilities for comprehensive two-dimensional gas chromatography (GC × GC), and a range of MDGC methodologies. Using an oscillating cryotrapping device [21] to achieve on-line trapping of compounds moving through a GC column, it is possible to sequentially focus and release solute to cause peaks to be modulated between two columns. Thus both GC × GC [22–26] and MDGC [27–29] methods can result from this mechanism.

Hyphenation of GC × GC with various detectors has been reported [30], with the flame ionization detector perhaps the most technically suitable with respect to performance and response times, but mass spectrometry remains the benchmark as the most desirable for multi-component identification [31,32]. The concern regarding the speed of acquisition for mass spectrometry with GC × GC (which is best accommodated by fast scanning time-of-flight MS) is relaxed somewhat in MDGC, since the chromatographic elution bands in MDGC are often much broader than in GC × GC. Hence slower scanning techniques such as quadrupole MS (qMS), ion-trap MS and isotope ratio MS (IRMS) can be used with confidence. Note however that qMS [33], ion-trap MS [34] and IRMS [35] have been used with GC × GC.

The preponderance of MS methods in GC analysis is a recognition of the vastly improved information content of GC/MS methods compared with the analogous non-spectroscopic method [36,37]. The capability of techniques such as GC/MS–MS to provide unique identification of (known) compounds in a very complex matrix is unsurpassed, since the matrix can effectively be made 'transparent', with the analytical response corresponding to just the single target compound.

Notwithstanding the philosophical arguments of the role of GC/MS–MS against GC × GC/MS [38], especially where unknown compounds arise, it is acknowledged that MS cannot solve all detection specificity problems, especially in the case of isomers. Thus we have sought to understand the role and utility of other spectroscopic techniques for absolute identification of compounds. In this respect, the best known routine tool for identification of organic compounds – nuclear magnetic resonance (NMR) spectroscopy – has recently been demonstrated by Albert and co-workers as an on-line tool for GC analysis, although the results are preliminary and the technical demands are substantial [39,40]. A unique implementation of micro-scale preparative MDGC with off-line NMR based upon the oscillating cryotrap, in conjunction with a microfluidic DS device, has recently been described and applied to the analysis of geraniol in a complex mixture of essential oils [41], and discrimination of methylnaphthalene isomers in a crude oil sample [42]. The latter application was significant because the compounds were present at natural abundance with relatively low levels (ca. 0.2–0.3%) in the mixture, and the sample was not subjected to any prior separation in order to isolate or concentrate the target compounds.

In this contribution we report an extension to the above studies using the micro-scale preparative MDGC system involving isolation of pure compounds followed by off-line NMR spectroscopy for analysis of the compounds that were not characterised by NMR in the former study [41]. These components were present at natural abundance in the essential oils, rather than being spiked into the complex oil mixture. In addition, the capabilities of using the same system for multiple component collections into the single trapping device are described. Whilst the use of NMR spectroscopy for multiple trapped compounds is not practical, it does demonstrate the ability to perform multiple trappings of selected components, with mass spectrometry used as the confirmation tool once the collected compounds are recovered. This method should serve a range of purposes, as explained herein.

2. Experimental

The present study extends our recently reported work [41] demonstrating the prep-MDGC technique with off-line NMR spectroscopy applied to the analysis of geraniol. In this case, selections of other compounds were chosen from the essential oil matrix to continue the prior work with a series of different tests.

2.1. Samples and Solvents

Sample solutions for GC separations were prepared using *n*-hexane (Pestanal®, ≥95%; Riedel-de-Haën, Seelze, Germany). Reference standards of geraniol ((2*E*)-3,7-dimethylocta-2,6-dien-1-ol, ≈98%; Aldrich Chemical Co, St Louis, MO), linalyl acetate (3,7-dimethylocta-1,6-dien-3-yl acetate; Australian Botanical Products (ABP), Hallam, Australia), and carvone (2-methyl-5-prop-1-en-2-yl-cyclohex-2-en-1-one; ABP) were used for method development and for confirming peak identification. Note that an authentic pure sample of menthol (5-methyl-2-propan-2-yl-cyclohexan-1-ol) was not used. Deuterated chloroform ($CDCl_3$, 99.8% stabilised with silver and 0.1% deuterated pyridine; Cambridge Isotope Laboratories, Andover, MA) and deuterated methanol (CD_3OD, UVAsol 99.8%; Merck, Darmstadt, Germany) were used for NMR spectroscopy.

A mixture of peppermint, spearmint and lavender essential oils (ABP; 1.0%, v/v each in hexane), spiked with geraniol (1.1 mg/mL) was used as a complex matrix to demonstrate the resolution and isolation of linalyl acetate, carvone and menthol using the prep-MDGC method. Chemical structures of the target compounds are given in Fig. 1.

2.2. Heart-cut multidimensional gas chromatography

The MDGC system was the same as reported previously [41,42], and is reproduced in Fig. 2. Here we provide a brief summary of the main components. Two capillary columns (^{1}D; ^{2}D) with

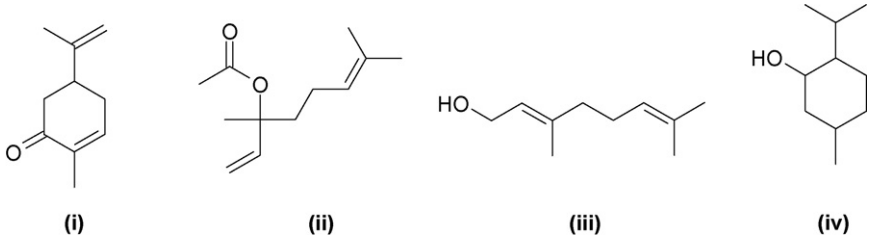

Fig. 1. Structures of (i) carvone (C), (ii) linalyl acetate (LA), (iii) geraniol (G), and (iv) menthol (M).