Chromatographia (2019) 82:377–398
https://doi.org/10.1007/s10337-018-3649-3

REVIEW

Multidimensional Gas Chromatography in Essential Oil Analysis. Part 1: Technical Developments

Leo Lebanov[1] · Laura Tedone[1] · Massoud Kaykhaii[2] · Matthew R. Linford[3] · Brett Paull[1]

Received: 18 June 2018 / Revised: 23 October 2018 / Accepted: 25 October 2018 / Published online: 31 October 2018

Abstract

Multidimensional gas chromatography (MDGC) is now established as a technique which can help resolve most of the co-elution problems presenting with conventional gas chromatography for highly complex samples. Essential oils (EOs) are often used in the optimisation and development of novel MDGC methods and related technologies, to demonstrate and assess performance. In this review, recent trends and technical developments in the optimisation of MDGC, modulation and system configuration, pertinent and applied to EO analysis, will be critically discussed. Optimisation of MDGC will be discussed with reference to different column configurations, modulation periods and detection. Attention is given to novel modulation systems, development of multiplex MDGC systems and new approaches which combine heart-cut and comprehensive modes within one system. A section of this review will be dedicated to the preparative application of the MDGC and its application in the isolation of less abundant compounds from complex EO matrices.

Keywords Multidimensional gas chromatography · Essential oil · Heart-cut multidimensional gas chromatography · Comprehensive multidimensional gas chromatography · Enantioselective multidimensional gas chromatography · Optimisation · Mass spectrometry · Multiplex dual-column systems · Preparative multidimensional gas chromatography

Introduction

Essential oils (EOs) are mixtures of volatile and semivolatile compounds obtained by hydrodistillation, steam distillation, dry distillation, or by some suitable cold-pressing method such as in the case of citrus oils. Due to the volatility and complexity of most natural oils, gas chromatography (GC) is the most widely used technique for EO separation, identification, and quantitative characterisation [1]. GC with a single column is still currently the method of choice for the routine analysis of EOs. However, due to the limited peak capacity of a single column, complete separation of all compounds by monodimensional GC is not always possible [2]. However, adding a second column with a different stationary phase, connected to the first dimension column (^{1}D) through a transfer device, can provide the necessary increased selectivity, and a significant increase in peak capacity and resolution. This technique is now well known as multidimensional gas chromatography (MDGC) and can be performed in either heart-cut (GC–GC) or comprehensive (GC × GC) modes [1, 2]. An essential element of MDGC is the efficient functioning of the transfer device, which acts to transfer fractions eluting from the first column onto the second dimension (^{2}D) column. During this process, these fractions can be focused into a narrow band and sent to ^{2}D column, which can provide increased sensitivity, and help with the identification of trace-level components.

In GC–GC, only selected, discrete fractions from the ^{1}D column separation are sent to the ^{2}D column [3], whilst in GC × GC, there is continuous modulation (fractionation) of the ^{1}D separation, where each fraction is transferred to the short, typically narrow-bore, ^{2}D column. Due to the

Published in the topical collection *Rising Stars in Separation Science*, as part of Chromatographia's 50th Anniversary Commemorative Issue.

✉ Brett Paull
brett.paull@utas.edu.au

[1] Australian Centre for Research on Separation Science (ACROSS), School of Natural Sciences, University of Tasmania, Private Bag 75, Hobart 7001, Australia

[2] Department of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan, University Boulevard, Zahedan 98135-674, Iran

[3] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA

separation of the whole sample on both columns, GC × GC offers an increase of overall resolution, total peak capacity and selectivity [4]. In terms of EO analysis, compared to GC–GC, GC × GC can analyse the whole sample on both columns in a single analysis. Therefore, in the last 10 years, GC × GC has been a popular choice for advanced EO characterisation [1, 5]. Several general reviews have been published in recent years focusing on advances in MDGC [1–4, 6–18], some of which have in their scope the application of MDGC in EO analysis [1, 4, 7, 8, 10, 13, 15, 18].

Technical innovations continue to generate new capabilities in MDGC, that act to increase the separation power of this technique. In addition, ongoing optimisation of current systems also continues to provide increasing performance. In this review (Part 1 of a two-part series), we will limit the scope to the technical advances in MDGC affecting EO characterisation over the past 10 years. All the instrumental parameters—including size and type of columns, modulators, and detectors will be critically discussed, as all are important components within an optimised MDGC method. In addition, systems that combine comprehensive and heart-cut modes, multiplexed systems with dual columns in one of the two dimensions, and MDGC applied for preparative purposes, are also reviewed. The application of MDGC in the characterisation of EOs analysis will not be discussed herein, as this will be covered within the second part of this mini review series.

MDGC Applied to Essential Oil Characterisation

To discuss MDGC separations of EOs, it is first necessary to understand the general nature of EOs. EOs typically range from moderate to highly complex mixtures and contain classes of organic compounds of varying volatility. These compounds, products of the secondary metabolism processes within the plants [7, 8, 10, 13], are mainly terpenes (mono-, sesqui- or diterpenes) and terpenoids, the latter having additional functional groups, like alcohol, aldehyde, ketone, ether, ester or acid. Many compounds have chiral centres, forming enantiomeric pairs, where one of them can be predominant or the only one present in an EO [7]. The diversity and varying degree of complexity of EOs is discussed in much greater detail the Part 2 of this mini review series, where the focus is on MDGC applications.

GC separations, as performed upon a single column, predominantly reflect two fundamental characteristics of the column, its selectivity and efficiency. Column efficiency contributes to peak capacity, a theoretical maximum value which can be defined as the number of peaks which can be separated, at a given minimum resolution (typically $R_s = 1$) across the entire chromatogram. For a standard 30 m × 0.25 mm ID GC column, peak capacity is typically ~ 400. However, for most real samples the compounds rarely elute as evenly distributed peaks across the entire chromatogram, but rather more often elute as peak clusters and co-eluting peaks, separated by windows of empty baseline [18]. To predict the number of observable peaks and to statistically describe overlap in 1D and 2D chromatographic system, Giddings and Davis postulated statistical-overlap theory, which defines the probability of single peak formation as $p = e^{-2m/n}$, where an m component mixture is analysed by a chromatographic system with maximum number of components that can be separated at certain resolution, defined as n [19, 20]. Therefore, peak capacity of the system needs to greatly exceed the number of components in the mixture, $\alpha = m/n$ with $\alpha << 1$ [21, 22]. For the EOs, with couple of hundreds of compounds this means that it is necessary for introduction of ^{2}D column to increase peak capacity of the chromatographic system.

The correct choice of column chemistry (selectivity) substantially helps in this regard and for relatively simple EOs, proper selection of stationary phase can directly provide the overall resolution required [18]. However, for the more complex EOs, where the individual components can number several hundred, the solution lies in applying a multidimensional approach [3, 18]. Here overall peak capacity is equal to the product of the peak capacity achieved within each dimension, such that theoretically at least (and provided orthogonal selectivity between columns), an order of magnitude or more of an increase in peak capacity can be achieved [15]. The actual mode of MDGC applied—heart-cut or comprehensive—as well as the choice of modulator and modulation period, in the case of GC × GC, may depend on the specific application, herein the complexity and nature of the EO.

Each of the above options, together with related conditions and parameters, technologies and capabilities will be explained in detail within this review. As a reference Tables 1 and 2 are provided as an upfront summary of the MDGC configurations, columns, transfer devices and detectors applied to EO analysis over the past decade.

Off-line Pre-separation Using Liquid Chromatography

Despite the remarkable resolving power of MDGC, in some cases a fractionation step prior to the MDGC separation has been applied, to decrease the complexity of the EOs and further improve overall resolution. To our knowledge, in the past 10 years which align with the scope of this review, this has been done only by HPLC. In this scenario, HPLC is used as an orthogonal technique to GC, performing polarity-based chemical-class separations. In this way, a sample for analysis using GC becomes less complex and more homogenous, allowing for the GC separation of one or a few 'classes' of

Table 1 GC×GC methods applied over the past 10 years in essential oil analysis

Plant species	^{1}D column stationary phase	^{2}D column stationary phase	Modulator	Detector	References
Piper regnellii (Miq.)	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[5]
Artemisia annua L.	(100%) Dimethylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[23]
Citrus auranthium L. or *Citrus*×*sinensis* (L.) Osbeck	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[24]
Polygonum minus Huds.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic dual jet	FID, TOFMS	[25]
Ocimum basilicum var. thyrsiflora	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	LMCS	FID	[26]
Ocimum basilicum L.	Polyethylene glycol	(100%) Dimethylpolysiloxane			
Chrysopogon zizanioides L.	(5%) Phenyl polysilphenylenesiloxane	Polyethylene glycol	LMCS	FID, qMS	[27]
	Polyethylene glycol	(100%) Dimethylpolysiloxane			
Cananga odorata [Lam.] Hook f. and Thomson	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[28]
Rosmarinus officinalis L.	(5%) Phenyl (95%) methylpolysiloxane	Trifluoropropyl	LMCS	FID	[29]
Piper nigrum L. *Thymus vulgaris* L. *Cuminum cyminum* L.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane		qTOFMS	
Notopterygium incisum Ting ex H.T. chang (Qianghuo in Chinese)	(100%) Dimethylpolysiloxane	Polyethylene glycol	Cryogenic quadruple jet	TOFMS, FID	[30]
Chrysopogon zizanioides L.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic dual jet	MS, FID	[31]
Aniba rosaeodora Ducke	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic quadruple jet	qMS	[32]
Aniba rosaeodora Ducke	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic quadruple jet	qMS	[33]
Manekia obtuse Miq *Piper cubataonum* C.DC.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic quadruple jet	qMS	[34]
Ilex paraguariensis A. St. Hil.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[35]
Angelicae sinensis (Oliv.) Diels	(5%) Phenyl (95%) methylpolysiloxane	(14%) Cyanopropyl-phenyl methylpolysiloxane	Not stated	HR-TOFMS	[36]
Aquilaria malaccensis Lamk	Silphenylene polymer	Polyethylene glycol	Cryogenic dual jet	qMS	[37]
Chrysanthemum morifolium Ramat.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Not stated	TOFMS	[38]
Citrus reticulata Blanco	Low polarity	(50%) Phenyl polysilphenylenesiloxane	Cryogenic quadruple jet	HR-TOFMS	[39]
Cynara scolymus L.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic dual jet	qMS	[40]
Pandanus fascicularis Lam.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Not stated	TOFMS	[41]
Hedychium coronarium J. Koenig	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic dual jet	TOFMS	[42]
Eucalyptus dunnii Maiden	(5%) Phenyl polysilphenylenesiloxane	Polyethylene glycol	LMCS	qMS, TOFMS	[43]
Citrus limon L. *Eucalyptus globulus* L.	(100%) Polydimethylsiloxane	Polyethylene glycol	Cryogenic dual jet	TOFMS	[44]

Table 1 (continued)

Plant species	^{1}D column stationary phase	^{2}D column stationary phase	Modulator	Detector	References
Citrus × *sinensis* (L.) Osbeck and *Citrus bergamia* (Risso)	Silphenylene polymer	Polyethylene glycol	Cryogenic dual jet	qMS	[45]
Various citrus EOs	Silphenylene polymer	Polyethylene glycol	Cryogenic dual jet	qMS, FID	[46]
Citrus deliciosa Ten. essential oils (green, yellow, red, and Mexican)	Silphenylene polymer	Polyethylene glycol	Cryogenic dual jet	qMS	[47]
Kunzea ambigua (Sm.) Druce	1,4-Bis(dimethylsiloxy) phenylene dimethylpolysiloxane	Polyethylene glycol	Cryogenic dual jet	FID	[48]
Rosa damascena Miller	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Flow	FID	[49]
	2,3-Di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin	Polyethylene glycol			
Mentha spicata L.	Diethyl-*tert*-butylsilyl-β-CD	Polyethylene glycol	Flow	FID	[50]
Artemisia annua L.	Silphenylene polymer	(35%) Diphenyl (65%) dimethylsiloxane	Flow	qMS	[51]
Lavandula spica L.	(5%) Phenyl polysilphenylenesiloxane	Polyethylene glycol	LMCS	FID	[52]
Melaleuca alternifolia (Maiden & Betche) Cheel	(5%) Phenyl polysilphenylenesiloxane	Polyethylene glycol	Valve	FID	[53]
Origanum onites L.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[54]
Rosmarinus officinalis L.	Polyethylene glycol	(5%) Phenyl (95%) methylpolysiloxane	Cryogenic dual jet	TOFMS	[55]
Chrysopogon zizanioides (L.)	(100%) Dimethylpolysiloxane	Polyethylene glycol	Flow	FID, MS	[56]
Mentha × *piperita* L. *Mentha spicata* L.	Polyethylene glycol	(86%) Dimethylpolysiloxane (7%) phenyl (7%) cyanopropyl			
Lavandula angustifolia Mill. *Lavandula angustifolia* Mill. × *Lavandula latifolia* Medik	(100%) Dimethylpolysiloxane	Polyethylene glycol	Flow	FID, MS	[56]
	Polyethylene glycol	(86%) Dimethylpolysiloxane (7%) phenyl (7%) cyanopropyl			
Mentha × *piperita* L., *Mentha arvensis* L., *Mentha spicata* L., *Mentha* × *gentilis* L.	(5%) Phenyl (95%) methylpolysiloxane	(86%) Dimethylpolysiloxane (7%) phenyl (7%) cyanopropyl	Cryogenic dual jet	MS, FID	[57]
Lavandula angustifolia Mill. × *Lavandula latifolia* Medik	(CSP) diluted at 30% in PS086 (DiEtβCD)	(86%) Dimethylpolysiloxane (7%) phenyl (7%) cyanopropyl	Cryogenic dual jet	FID, qMS	[57]
Atractylodis macrocephalae	(5%) Phenyl (95%) methylpolysiloxane	(14%) Cyanopropylphenyl methylpolysiloxane	Not stated	TOFMS	[58]
Leonotis leonurus (L.) R. Br.	Polyethylene glycol	1,4-Bis(dimethylsiloxy)phenylene dimethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[59]
Sassafras albidum (Nutt.) Nees	(5%) Phenyl polysilphenylenesiloxane	(90%) Cyanopropyl polysilphenylenesiloxane	Cryogenic quadruple jet	TOFMS	[60]
Rosmarinus officinalis L.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Cryogenic quadruple jet	FID	[61]
Chaihu Shugan San	1,4-Bis(dimethylsiloxy) phenylene dimethylpolysiloxane	(50%) Phenyl polysilphenylenesiloxane	Cryogenic (not stated the type)	qMS	[62]

Table 1 (continued)

Plant species	^{1}D column stationary phase	^{2}D column stationary phase	Modulator	Detector	References
Origanum vulgare L. *Rosmarinus officinalis* L.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Valve	qMS, FID	[63]
Artemisia umbelliformis Lam.	(5%) Phenyl (95%) methylpolysiloxane	(86%) Dimethylpolysiloxane (7%) phenyl (7%) cyanopropyl	Cryogenic dual jet	MS, FID	[64]
Melaleuca alternifolia (Maiden & Betche) Cheel	Dimethyl polysiloxane Polyethylene glycol	(50%) Phenyl (50%) dimethylpolysiloxane	Flow	qMS	[65]
Citrus reticulata Blanco *and Mentha spicata* L.	Silphenylene polymer	1,12-Di(tripropylphosphonium) dodecane bis (trifluoromethanesulfonyl)imide	Flow	QqQMS	[66]
Panax ginseng C. A. Mey	(5%) Phenyl (95%) methylpolysiloxane	(14%) Cyanopropyl phenylmethylpolysiloxane	Cryogenic quadruple jet	TOFMS	[67]
Lonicerae japonicae Thunb.	(5%) Phenyl (95%) methylpolysiloxane	(50%) Phenyl (50%) dimethylpolysiloxane	Not stated	TOFMS	[68]
Artemisia arborescens L.	Silphenylene polymer	(50%) Phenyl (50%) dimethylpolysiloxane	Flow	qMS	[69]
Rosa damascena Miller	2,3-Di-*O*-ethyl-6-*O*-*tert*-butyl-dimethylsilyl-β-cyclodextrin	Polyethylene glycol	Flow	FID, qMS	[70]
Lavandula angustifolia Miller	2,3-Di-*O*-ethyl-6-*O*-*tert*-butyl-dimethylsilyl-β-cyclodextrin	Polyethylene glycol	Flow	FID, qMS	[71]
	2,3,6-Tri-*O*-methyl-β-cyclodextrin	Polyethylene glycol			
Mentha spicata L.	Diethyl-t-butylsilyl β cyclodextrin	Polyethylene glycol	Flow	FID	[72]

compounds at a time [13, 46]. Several authors have applied this hyphenated technique to citrus EOs. Tranchida et al. [45] used this concept for a more thorough chemical profiling of sweet orange and bergamot EOs (Fig. 1). The HPLC pre-separation gave two fractions: hydrocarbons and oxygenated compounds. Subsequent GC×GC analysis with a quadrupole mass spectrometer (qMS) as the detector, allowed the identification of many compounds reported for the first time. Figure 2 shows one of the reported chromatograms from this work. However, from this figure it is clear that in the sample shown, which contains the pre-fractionated sesquiterpenes, the peaks elute with similar retention times in ^{2}D, suggesting that standard monodimensional GC would have provided essentially equal resolution for this fraction. Greater separations were achieved by applying GC×GC to the fractions containing oxygenated compounds Fig. 3a–d. Later, Zoccali et al. [46] used HPLC off-line to obtain fractions containing sesquiterpenes from several cold-pressed citrus EOs, which were then separated and identified by GC×GC. Compounds found in all samples can be used as general markers for citrus oils, while the ones identified only in a specific citrus EO can be used as the markers to distinguish different chemotypes [47].

Column Configuration

In the substantial majority of publications on EO analysis by MDGC in the last 10 years, the column used in the first dimension (^{1}D) has been non-polar, either (100%) dimethylpolysiloxane or (5%) diphenyl (95%) dimethyl polysiloxane, typically with dimensions of 30 m×0.25 mm×0.25 µm, while in the second dimension (^{2}D) (50%) diphenyl (50%) dimethylpolysiloxane or polyethylene glycol (PEG) columns were used (Tables 1, 2). The column configuration with a non-polar column in ^{1}D and a more polar ^{2}D allows separation in the ^{1}D mainly due to dispersion interaction and in the second dimension according to the polarity of the components. Another reason for the application of this arrangement is that linear temperature programmed retention indices (LTPRI) obtained from the non-polar column are more rugged [65], providing higher similarity to the LTPRIs from data libraries. Methods for the determination of LTPRIs on the ^{2}D column fall within the scope of the Part 2 of this mini review series.

The chemical nature and column manufacturing process for polar phases, involving different levels of cross-linking, can produce a higher variation in the resultant stationary phase properties. This has been particularly noted for

Table 2 GC–GC methods applied in the past 10 years in essential oil analysis

Plant species	[1]D column stationary phase	[2]D column stationary phase	Transfer device	Detector	References
Lavandula spica L.	(5%) Phenyl polysilphenylenesiloxane	Polyethylene glycol	Deans switch	FID	[52]
Theobroma cacao L.	Polyethylene glycol	Heptakis (2,3-di-*O*-methyl-6-*O*-tertbutyldimethylsilyl)-b-cyclodextrin (25%)	Deans switch	FID	[73]
Pistacia lentiscus L.	(5%) Phenyl (95%) methylpolysiloxane	Diethyl-*tert*-butylsilyl-β-cyclodextrin	Deans switch	FID, qMS	[74]
10 different proprietary amounts of botanical and citrus oils used in the formulation of gin	Polyethylene glycol	(50%) Phenyl (50%) dimethylpolysiloxane	Cryogenic trapping system	FID	[75]
Protium heptaphyllum (Aubl.) Marchand	(5%) Phenyl (95%) methylpolysiloxane	Not stated	Deans switch	qMS	[76]
Lavandula spica L.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	LMCS	FID, qMS	[77]
Mentha×*piperita* L. *Lavandula spica* L.	Silphenylene polymer	Ionic liquid (IL) column	LMCS/deans switch	FID	[78]
Melaleuca alternifolia (Maiden & Betche) Cheel	(50%) Phenyl (50%) dimethylpolysiloxane	2,3,6-Tri-*O*-methyl derivative of β-cyclodextrin	Deans switch	qMS	[79]
Melaleuca alternifolia (Maiden & Betche) Cheel	Silphenylene polymer	Polyethylene glycol	Deans switch	qMS, FID	[80]
Santalum album L. *Santalum spicatum* (R. Br.) A. DC. *Santalum austrocaledonicum* Vieill.	Silphenylene polymer	Polyethylene glycol	Deans switching	qMS,FID	[81]
Coriandrum sativum L. *Humulus lupulus* L.	(5%) Phenyl (95%) methylpolysiloxane	Polyethylene glycol	Deans switch	FID, sniffing port	[82]
Citrus limon (L.) Osbeck	Polyethylene glycol	(5%) Phenyl (95%) methylpolysiloxane	Deans switch (CFT)	MS, FID	[83]
Citrus aurantifolia Swingle and *Citrus latifolia* Tanaka	Silphenylene polymer	Diethyl-*tert*-butyl-silyl β-cyclodextrin	Deans switch	qMS	[84]
Mentha piperita L. *Carum carvi* L.	(5%) Phenyl (95%) methylpolysiloxane	Chirasil-β-Dex	Deans switch	Ion-trapMS	[85]
Citrus limon (L.) Obreck. and *Citrus reticulata* Blanco petitgrain EO	(5%) Phenyl (95%) methylpolysiloxane	Diethyl-*tert*-butil-silyl β-cyclodextrin	Deans switch	qMS	[86]
Authentic essential oils from *Origanum vulgare* L., *Satureja* L. *Thymus vulgaris* L. and *Foeniculum vulgare* Mill.	(5%) Phenyl (95%) methylpolysiloxane	Heptakis-(2,3-di-*O*-methyl-6-*tert*-butyldimethylsilyl)-β-cyclodextrin (30%)	Deans switch	Ion-trap MS	[86]
	(5%) Phenyl (95%) methylpolysiloxane	Heptakis-(2,3-di-*O*-methyl-6-*tert*-butyldimethylsilyl)-β-cyclodextrin (30%)			
	(5%) Phenyl (95%) methylpolysiloxane	Heptakis-(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (50%) in OV 1701 (50%)			

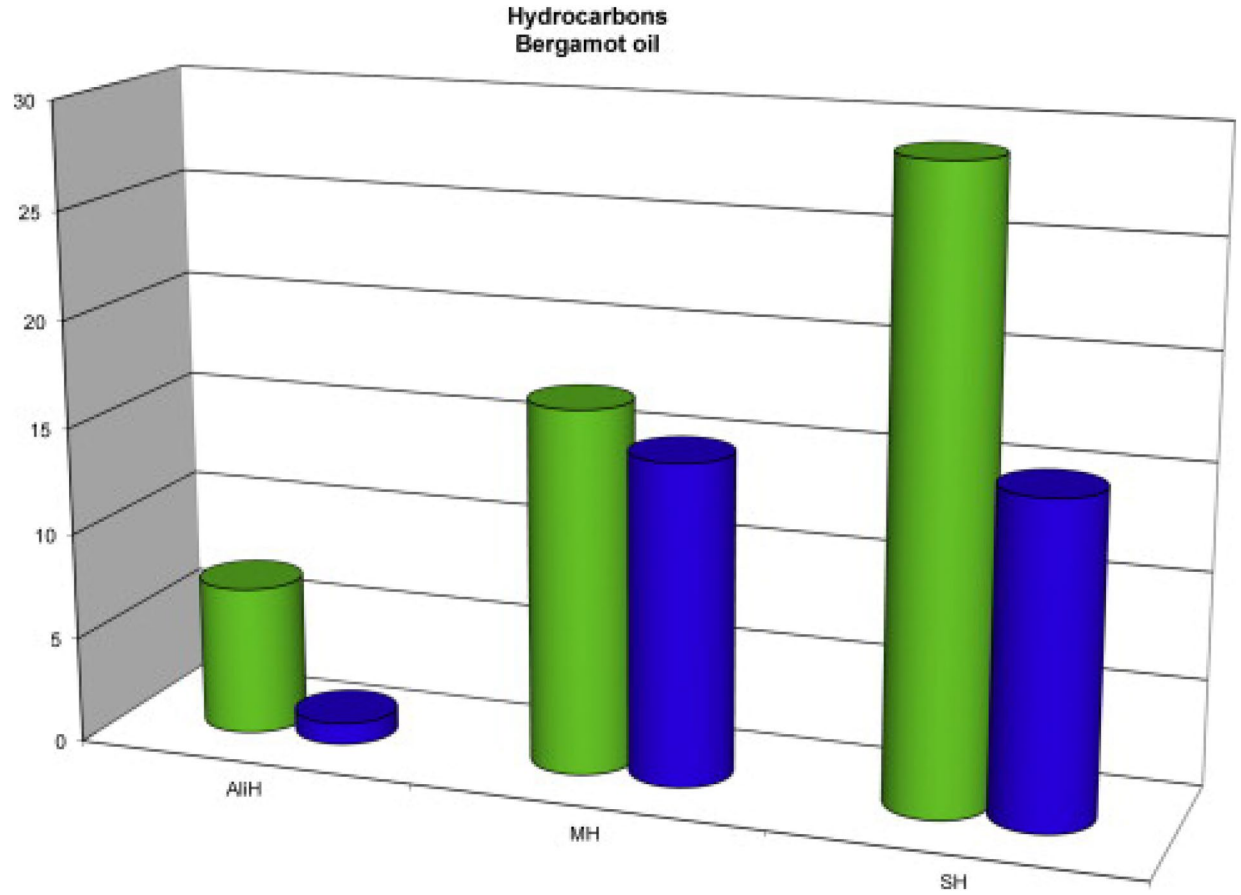

Fig. 1 Number of bergamot oil hydrocarbons identified with GC×GC/qMS (green bar) and GC/qMS (blue bar). *AliH* aliphatic hydrocarbons, *MH* monoterpenes, *SH* sesquiterpenes. Reproduced with permission from [45]

polyethylene glycol-type (PEG) columns [87, 88]. These observations are in agreement with those made by Morrison et al. [49], whose evaluation of polar columns revealed that (50%) diphenyl (50%) dimethyl polysiloxane columns were more thermally stable compared to wax-based stationary phases, and less prone to degradation and change of performance with use. They also showed that PEG columns were well-suitable for the separation of polar components in EOs [49], and thus, the column of choice in the ^{2}D (Tables 1, 2). However, it is important to also comment that the selection of a polar column for the ^{2}D can lead to potential wrap-around effects, due to the high retention of most polar compounds, leading to a problem in their full separation and characterisation [65].

In their 2015 study, Cordero et al. [56] evaluated five different column and system configurations using a volatile model mixture (Table 3). Each setup was evaluated using several performance parameters to characterise GC×GC separations, namely re-injection pulse width (σ^2_i), net separation measure ($S_{GC\times GC}$), modulation ratio (M_R), used separation space, and corresponding pixel-based area ratios (pixel counts). The initial column configuration evaluated was the most commonly applied combination, also being the manufacturer's suggested configuration (Table 3), exhibiting very low ^{1}D average carrier velocity resulting in longer analysis times and limited separation power. To increase the carrier gas velocity, a shorter column with smaller internal diameter and film thickness was used keeping the same phase ratio. Co-elution of several peaks was resolved by increasing the column loadability, increasing the film thickness, and increasing the length of the ^{2}D column. Increasing the orthogonality of the column system using a PEG column in ^{2}D provided a clear improvement on the performance, with this configuration having the highest value of utilised area and separation space, whilst at the same time producing the narrowest peaks and having the M_R value above three (Table 3), which, according to the authors is required to obtain a good degree of confidence for area determination for trace solutes [56]. Thus, this setup (Configuration 4, Table 3) was used for the analysis of a very complex vetiver EO. Using Configuration 5, a polar column in ^{1}D and a mid-polar in ^{2}D was applied, which led to a very different peak distribution in the 2D contour plot. Compared to Configuration 4, there was a significant increase in the $S_{GC\times GC}$,

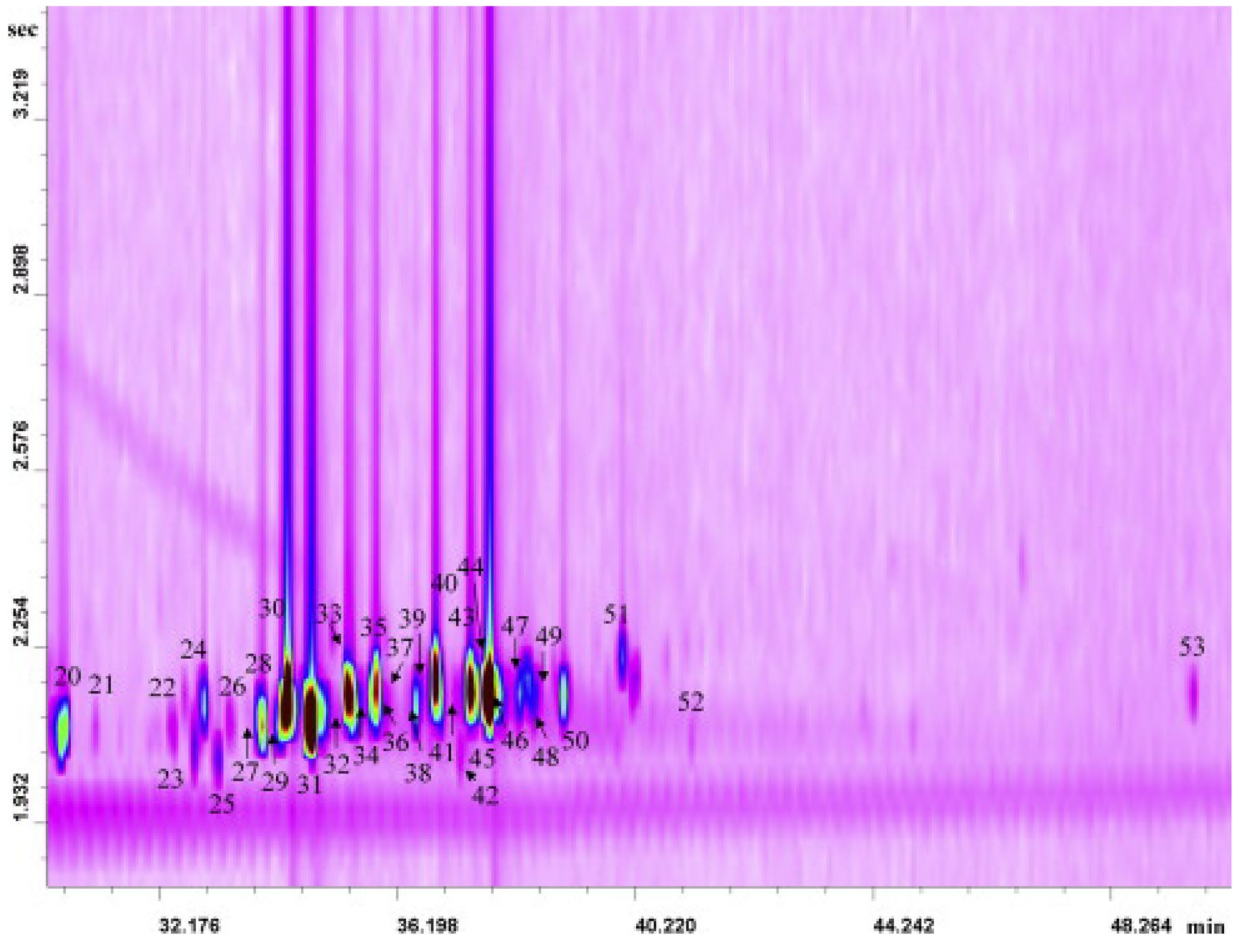

Fig. 2 GC × GC/qMS chromatogram of sesquiterpenes in bergamot essential oil. Reproduced with permission from [45]

with a narrower σ^2_i, M_R being above 3, and at the same time decreasing used separation space and area (see Table 3). This configuration was applied for the determination of mint and lavender EOs [56].

In the most efficient separations obtained, the dimensions of the ^{1}D column in the work of Cordero et al. were 10 m × 0.10 mm × 0.40 µm. However, columns used in the majority of studies published in the past 10 years were longer, with a larger internal diameter and thinner coating (30 m × 0.25 mm × 0.25 µm) (see Table 1). The advantage of the shorter column is the faster analysis, while the columns with a narrower diameter and thinner film thickness require more diluted samples or the application of a higher split ratio during injection. Ma et al. [23] performed a qualitative analysis of *Artemisia annua* L. using columns with higher retention factors and peak capacities, specifically DB-Petro 50 m × 0.20 mm × 0.5 µm, 100% dimethylpolysiloxane and DB-17ht 2.6 m × 0.10 mm × 0.1 µm (50%-phenyl) methyl polysiloxane (see Table 1). They tentatively identified 303 compounds, out of 700 peaks, with 33 having a relative abundance greater than 0.2%. Qin et al. [39] managed to characterise *Citrus reticulata* Blanco (Chenpi in Chinese, dry tangerine skin) pericarpium, a herbal medicine mostly used in Korea, China and Japan, using GC × GC/high resolution (HR)-time of flight mass spectrometry (TOFMS), with a 15 m × 0.25 mm × 0.25 µm low-polarity column in ^{1}D, and a shorter 1.0 m BPX50 column with standard coating thickness in the second dimension. In this way, the speed of analysis was increased, with excellent peak identification using the HR-TOFMS. Using a GC × GC/qMS with a 60 m-long column in the ^{1}D, Saucier et al. [40], managed to identify 130 compounds, out of which 109 were identified in the artichoke EO for the first time. As well as using most common column configurations, Namara et al. [75] and Robbat et al. [89] applied similar GC–GC approaches, with polyethylene glycol column in ^{1}D and a non-polar column in ^{2}D, to separate compounds present in juniper berry EOs, and EOs used for the development of gin, with the aim of building libraries of all compounds present.

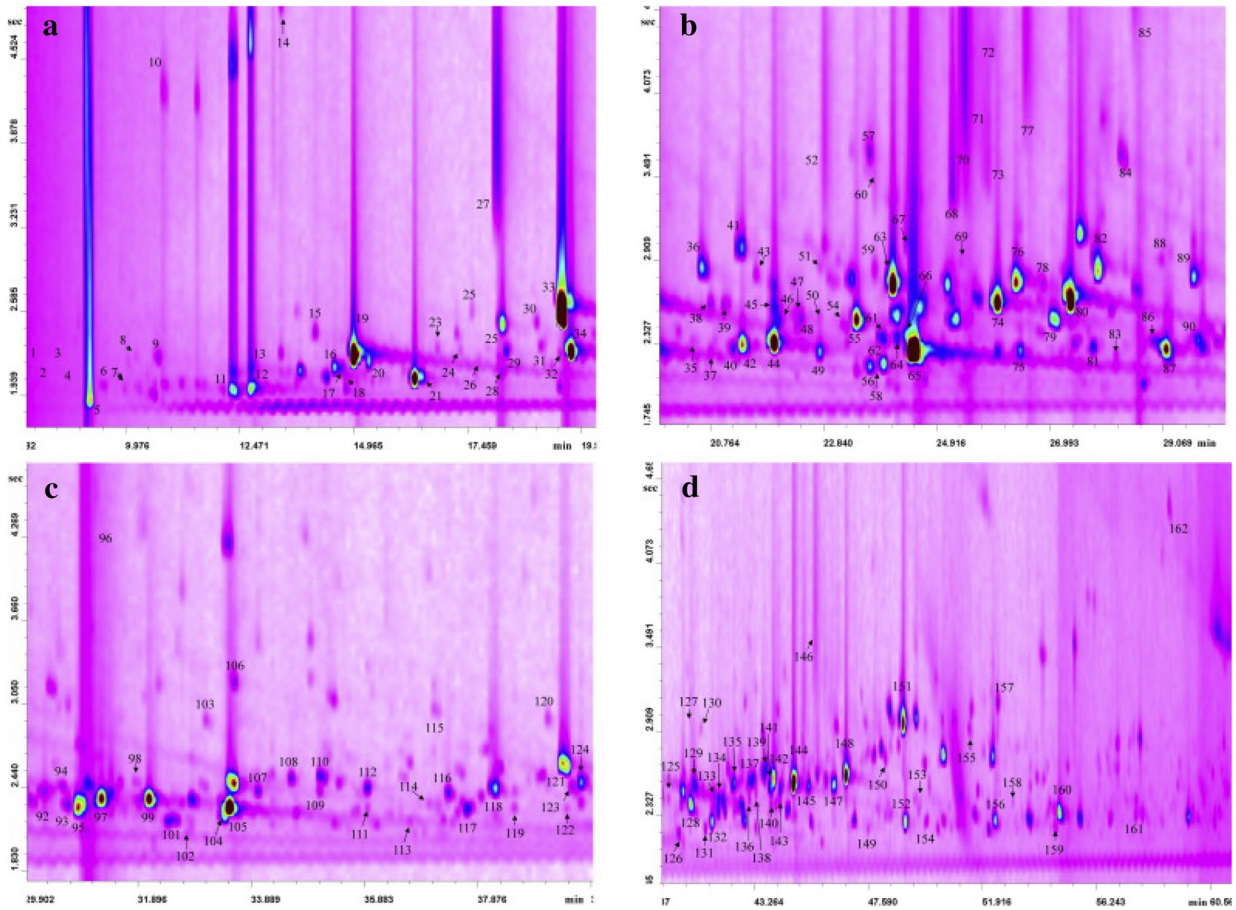

Fig. 3 GC × GC/qMS chromatogram of oxygenated compounds in orange essential oil. Reproduced with permission from [45]

Table 3 Column setting and configurations used by Cordero et al. [56] and the reported performance data

	Configuration 1	Configuration 2	Configuration 3	Configuration 4	Configuration 5
Column ^{1}D	SE52	SE52	OV1	OV1	PEG
Coating ^{1}D	95% Polydimethylsiloxane, 5% phenyl	95% Polydimethylsiloxane, 5% phenyl	100% Dimethylpolysiloxane	100% Dimethylpolysiloxane	Polyethylene glycol
Dimension ^{1}D (length × internal diameter r × film thickness)	30m × 0.25 mm × 0.25 µm	10 m × 0.10 mm × 0.10 µm	10 m × 0.10 mm × 0.40 µm	10 m × 0.10 mm × 0.40 µm	10. 0m × 0.10 mm × 0.10 µm
Column ^{2}D	OV-1701	OV-1701	OV-1701	PEG	OV-1701
Coating ^{2}D	86% Polydimethyl siloxane, 7% phenyl, 7% cyanopropyl	86% Polydimethyl siloxane, 7% phenyl, 7% cyanopropyl	86% Polydimethyl siloxane, 7% phenyl, 7% cyanopropyl	Polyethylene glycol	86% Polydimethyl siloxane, 7% phenyl, 7% cyanopropyl
Dimension ^{2}D (length × internal diameter × film thickness)	5.0 m × 0.25 mm × 0.2 µm	1.0 m × 0.10 mm × 0.1 µm	1.50 m × 0.10 mm × 0.1 µm	1.50 m × 0.10 mm × 0.1 µm	1.50 m × 0.10 mm × 0.1 µm
$\sigma^2_{\text{i last peak}}$	0.11 s	0.10 s	0.07 s	0.10 s	0.08 s
$S_{GC\times GC}$	8711	13,512	27,466	16,955	35,724
$M_{\text{R last peak}}$	13.98	8.13	3.96	3.36	3.91
Separation space used	0.66	0.95	0.74	0.98	0.75
Area used (pixel)	41,445	75,369	70,581	106,322	79,089

Chiral Columns

Chiral compounds are commonly present in the EOs and the determination of their ratios, defined as enantiomeric excess (EE), which is specific for the species and chemotype, can thus be used for the assessment of the purity and potential adulteration of the EOs. Limitations in classical enantioselective 1D GC to distinguish targeted chiral compounds from the other interfering peaks, has led to the more widespread application of enantioselective MDGC, where one of the columns has chiral selectivity. Due to the limited number of chiral compounds within the samples, which in some cases elute within a narrow elution window [74], application of GC–GC is the more common approach, compared to GC × GC (Table 2). In the last decade the most common configuration has placed the enantioselective column in ^{2}D, which allows the separation of compounds on the achiral ^{1}D column and then transfer of the targeted compounds to ^{2}D for the chiral separation (see Table 2) [79].

Application of the enantioselective column in second dimension of a GC × GC system can suffer from limited separation of chiral compounds on the short ^{2}D column. Alternatively, GC × GC with enantioselective column in ^{1}D requires that peaks should be sufficiently resolved to be determined in two or more different modulation periods, avoiding wrap-around, to obtain more reliable quantification. Separation must be optimised to obtain greater M_R, which means increased data density, and minimising the unresolved overlapped region, allowing better reconstruction of the ^{1}D peaks and better quantification of the enantiomeric pairs [79].

Wong et al. [79] applied GC–GC with an enantioselective column in the ^{2}D, delivering the separation of chiral compounds within 25 min. However, utilising GC × GC with the enantioselective column in the ^{1}D, the same chiral analysis was completed in under 20 min by optimising the column and oven temperature program. Further applications of enantioselective MDGC in the EO analysis will be covered in more detail explained in Part 2 [97].

Transfer Devices in GC–GC

To perfrom GC–GC analysis, a transfer device in the system needs to transfer selected fractions of the primary column effluent to the head of the ^{2}D column. For this purpose, multiport valves have long been used as transfer devices in GC–GC, where the Deans Switch, and variants thereof, are by far the most commonly used transfer device for heart-cutting. Transfer devices based upon the Deans Switch contain two components, one within the sample path and one outside of the sample path. The part on the sample path contains no moving part and is located inside of chromatography oven, while the other, which contains movable parts (normally a solenoid valve) is located outside the oven. A Deans Switch uses an auxiliary flow of carrier gas to control the transfer of effluent from the ^{1}D to ^{2}D columns [2].

There have been several transfer devices recently introduced for heart-cutting. Agilent have developed their capillary flow technology (CFT) [90], and both PerkinElmer [91] and SGE [92] have introduced microchip devices which combine individual switch components into single small deactivated microchip plates. Such microfluidic designs help eliminate previous chromatographic problems by maintaining inert surfaces which prevent peak tailing or loss of solutes. In addition, low thermal mass allows the Deans Switch to equilibrate to the oven ramp temperatures quickly, which helps reduce peak broadening [3, 4]. The CFT Deans Switch was applied by Wong et al. [79] for the enantioselective analysis of tea tree EO and by Sasamoto et al. [83] in a 1D/2D GC/MS system applied to the analysis of lemon EO. Shimadzu have introduced a multi-Deans switching technology [93], with a heated transfer line connecting the two columns and with deactivated internal surfaces, and pressure control applied to both sides. The system is said to provide improved retention time reproducibility, and allows multiple transfers of different fractions during a single run. Gerstel's multi-column switching approach [94] is based on positioning the exit of 1D column next to the entrance of the 2D column in a stream of auxiliary carrier gas, inside a glass cap [3, 4].

Development of Modulators

Modulation is the heart of GC × GC, providing accumulation and trapping, refocusing, and rapid release of the components eluting from the first column, providing for fast and efficient separations [63]. The most frequently applied modulators in GC × GC analysis of EOs (Table 1) are thermal, valve-based, and flow modulators (FM). Thermal modulators predominate, due to the fact that GC × GC systems with these modulators have higher sensitivity compared to the systems based upon flow modulators, despite their relative complexity. Advances in the technology of transfer devices in MDGC has been the topic of many previous reviews to which the reader is here directed [2–4, 6, 12, 16, 17].

Thermal Modulators

The most frequently used thermal modulators (TMs) in EO analysis are the cryogenic ones, longitudinally modulated cryogenic system (LMCS) and jet modulators (Table 1). LMCS is the oldest thermal modulator type, developed by Marriot et al. [95] and is able to increase sensitivity and peak capacity compared to conventional GC by trapping the peaks of interest using liquid CO_2 and then rapidly heating up the the portion of the column holding the trapped components, providing fast release of the focused peaks into the ^{2}D

column (Fig. 4A, B-a). The shortcomings of this system are the inability to trap very volatile species due to the relatively high trapping temperature of − 50 °C, plus the necessity of moving parts, making this system less robust. However, since EOs tend to have as their most volatile organic compounds the monoterpenes, the use of LMCS is still applicable for most EO analysis and so applied by many researchers (Table 1). Compared to LMCS, jet-based modulators use either carbon dioxide (Fig. 4B-b) or liquid nitrogen, and have simple valves as the only moving parts (Fig. 4B-c, d). Most commonly applied are those with 4 jets (Table 1; Fig. 4B-d). For the analysis of very volatile compounds, with boiling points lower than hexane, the application of liquid nitrogen for the trapping is necessary due to the significantly lower trapping temperature of − 160 °C [6, 12, 16]. A great disadvantage of this system is the high consumption of liquid N_2, up to the 100 L day^{-1}, making this technique very expensive [12, 16].

Valve-Based Modulators

Valve Modulators Advances in valve modulators have been described previously by Seeley [2]. Valve-based modulators began with diaphragm-based valve modulators to divert the effluent from the ^{1}D column to the head of a short ^{2}D column. The main limitations of these early systems were that only 10% of the primary effluent reached the ^{2}D column, which obviously decreased the sensitivity of the analysis, and a temperature limit of 175 °C. The temperature limit could lead to the condensation of less volatile compounds in the valve [2]. This limitation was overcome by the introduction of perfluoroelastomer-based O-rings, instead of temperature-sensitive ones used in the first designs, allowing reliable operation of the system up to 325 °C [16].

The advantage of valve modulators, compared to thermal modulators, is the efficacy in capturing very volatile compounds, ease of use, and no consumption of liquid nitrogen, which make valve modulators a much better choice for the analysis of volatile compounds in remote locations using portable systems [53]. Lidster et al. [53] utilised a rotary-valve modulated (Fig. 5) GC × GC system to analyse gasoline and tea tree EO.

Flow-Switching Modulators Flow-switching modulators (FMs) are based on collecting the ^{1}D effluent in a collection loop during the fill cycle, and then sending the collected fraction to the ^{2}D column by changing the direction of the auxiliary flow, during the flush cycle. FMs offer an advantage over thermal modulators, especially when applied in remote field operations, as there is no necessity for cooling by a cryogen [49, 50]. There have been reports by users of unsatisfactory performance because of difficulties in obtaining rugged modulation conditions [72]. However, it has been shown that by optimising the GC × GC system, the efficiency of FM can be increased. Application of a long narrow-bore column in the ^{1}D [72], increases the resistance and enables efficient stop-flow modulation. In addition, the application of a long wider bore column in the ^{2}D (e.g. 10 m × 0.32 mm × 0.20 μm) is common due to the high gas flow and the widths of injected chromatographic bands, normally associated with FM, which allows the use of lower pressure for flushing the loop having the flow exiting the chromatographic system compatible with some kind of MS detectors [18]. Thus, the correct optimisation of the column dimensions can increase the performance of the FM-based GC × GC system to provide satisfactory results [18].

In forward fill/flush (FFF) mode, a flow modulator uses a loop channel, positioned in between the two columns, to collect the effluent from ^{1}D during the fill cycle, and the loop is then flushed in the same direction onto the ^{2}D column [49]. FFF modulator has been found by users to give satisfactory results when concentrations of the target compounds do not differ significantly. Separation of highly abundant compounds in ^{2}D can lead to peak tailing and co-elution with less abundant compounds, which is also a common problem with thermal modulators. This effect has been overcome using a modified reverse fill/flush (RFF) flow modulator (Fig. 6), by introduction of a variable collection channel and a restrictor, which works as an outlet for the carrier gas through the collection channel during the fill cycle, and allows to reverse the flow direction during the flush cycle. This setup offers less band broadening in the ^{2}D and reduced overloading, without losing separation power and resolution [49]. RRF provides improved reproducibility of ^{1}D and ^{2}D peak retention times, with Cordero et al. reporting the highest value of variation being 6.58% (neomenthol) on the ^{2}D column [56].

Tranchida et al. [50] developed a new flexible loop-type FM with efficiency comparable to a cryogenic FM, showing comparable peak widths (4σ) of 250–300 ms in ^{2}D for (+)-β-pinene, isomenthone, menthol, and caryophyllene. However, the high gas flow associated with FM is a disadvantage for the flexible loop-type FM system (Fig. 7), especially when it is hyphenated with MS. Therefore, it was demonstrated that by using different restrictor lengths in the connections linking the modulator to the auxiliary pressure source, it was possible to increase the duration of re-injection and greatly reduce gas flows, down to 4 mL min^{-1}, to make the system compatible with MS detection [51]. By optimising the diameter of the modulation loop column preceding the ^{2}D column, and decreasing it to 0.10 mm, there was a reduction in the volume of the final segment of the loop leading to limited band broadening and a decrease in peak widths. Therefore, there was a significant increase in the resolution and decrease in the plate height of the system with FM [37].

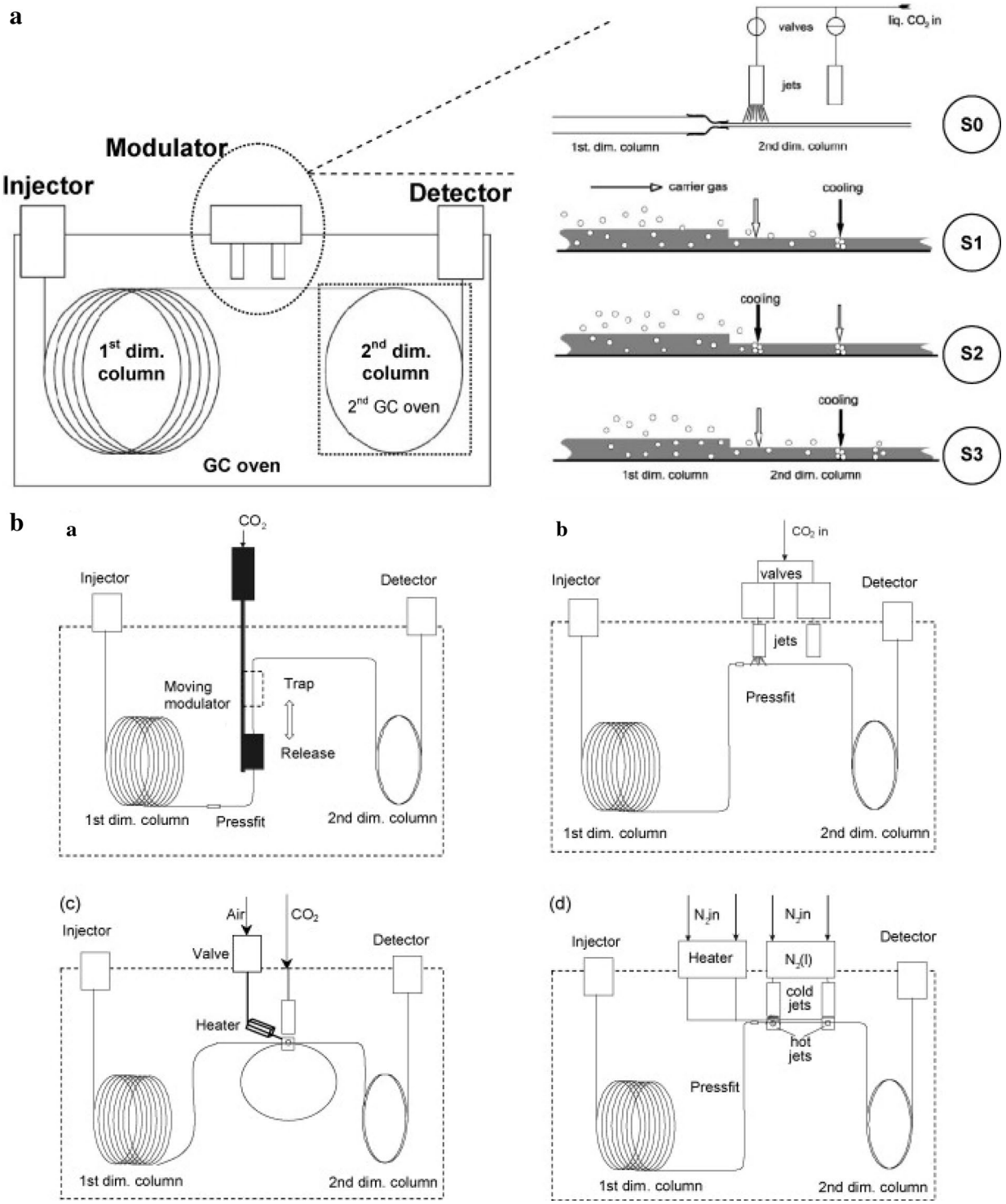

Fig. 4 **A** Schematic presentation of two-oven GC×GC system and explanation of cryo-modulation. Left: typical configuration. Right: (S0) general configuration of dual-jet cryogenic modulator. (S1) Right-side jet traps analytes eluting from ^{1}D column; (S2) right-side jet switched off, cooled effluent from ^{1}D column heats up rapidly and analyte pulse is released into ^{2}D column; simultaneously, left-side jet switched on to prevent leakage of ^{1}D column material; (S3) next modulation cycle is started. **B** Schematics of four cryotrap modulators: **a** LMCS; **b** dual-jet CO_2 modulator; **c** dual-jet N_2 cryo and **d** four-jet N_2 cryotrap. Reproduced with permission from [6]

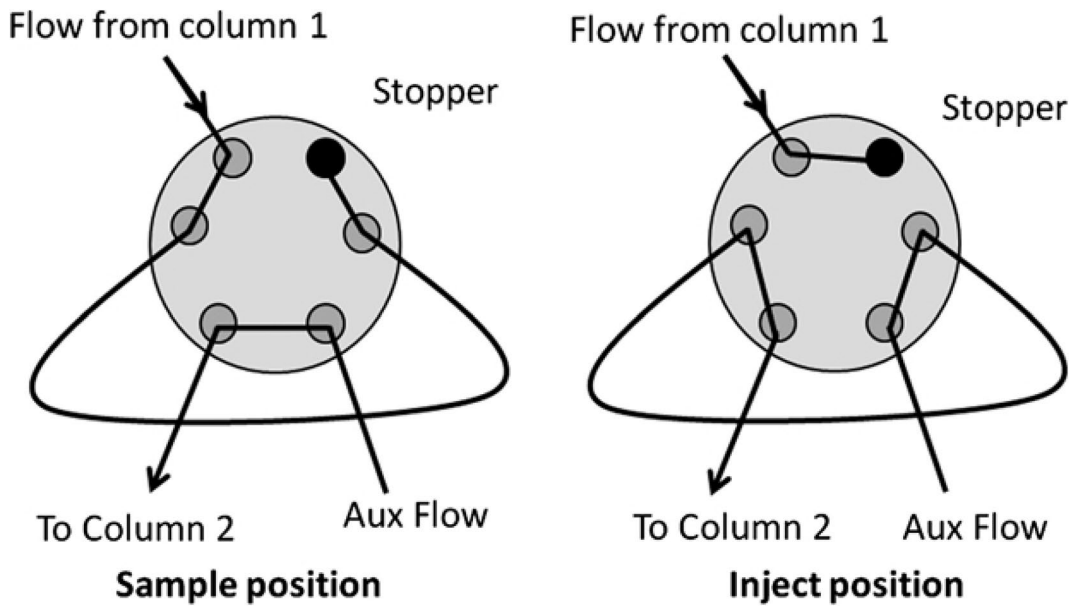

Fig. 5 The valve modulator design using 6-port, 2-way rotary valve, utilising a stopper to ensure that no sample is sent to waste. Reproduced with permission from [53]

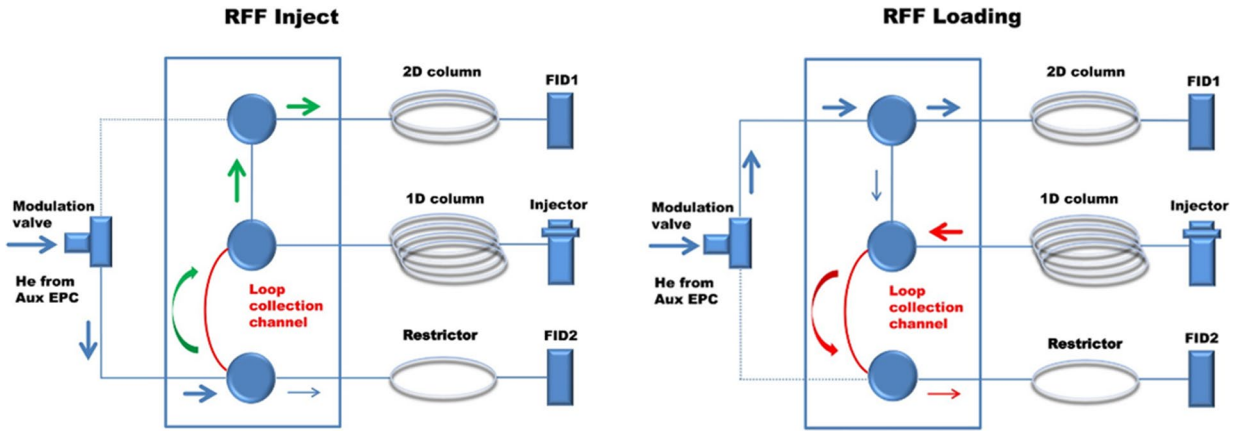

Fig. 6 Reverse fill/flush flow-modulated GC × GC system. Reproduced with permission from [49]

Modulation Period

Modulation period is one of the most important parameters to be optimised in GC × GC. Because of the typically short length of the ^{2}D column in GC × GC, a long modulation period can deliver a large number of compounds, which need to be separated on the ^{2}D column. Effluent coming from ^{1}D column, containing numerous compounds, can exceed the limited ^{2}D column peak capacity, leading to co-elution in the ^{2}D and decrease in already achieved resolution in the ^{1}D and in overall performance of the GC × GC system. Also, longer modulation period leads to lower M_R which is only good for screening or semi-quantitative analysis, where for the quantification is necessary M_R of at least 3 [79] (already explained in "Chiral columns"). However, if the modulation period is too short, some compounds retained longer on the ^{2}D column and do not elute from ^{2}D column before the next modulation cycle starts causing wrap-around [24]. Ideally the ^{2}D separation should be finished before the next injection of the subsequent fraction of the ^{1}D effluent [4]. In the majority of studies performed over the last 10 years, the reported value for a modulation period was 6 s, and the values varied over the range of 2–10 s. In GC–GC, the time of the heart-cut is similarly important, as it is necessary that a whole section of interest be transferred quantitatively to

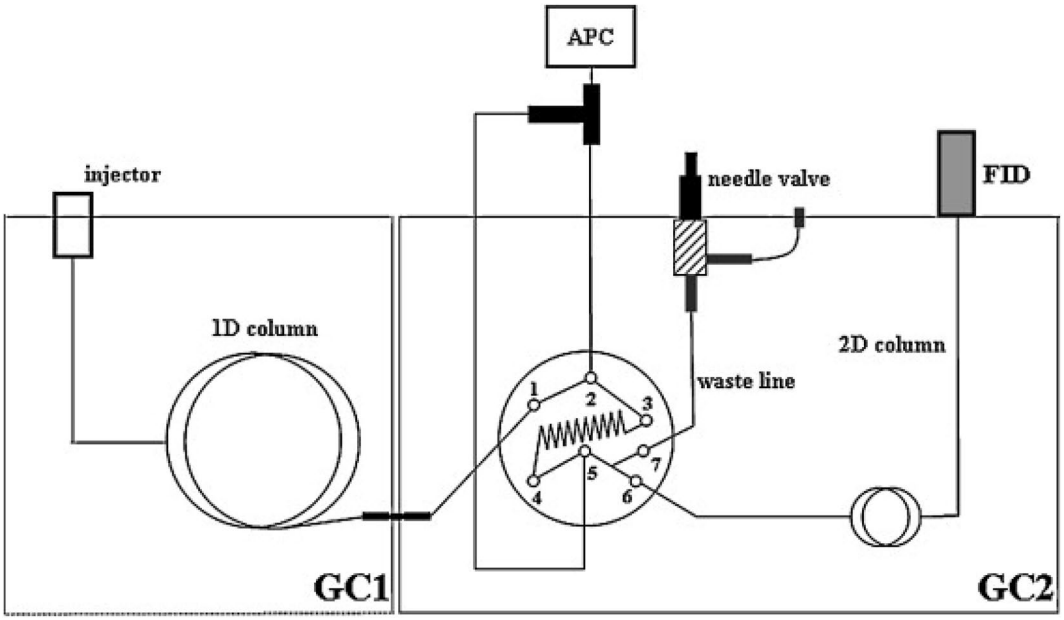

Fig. 7 Loop-type flow modulator. Reproduced with permission from [50]

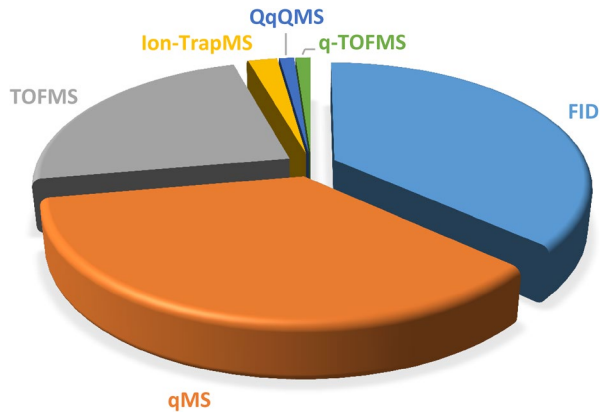

Fig. 8 Variety and relative occurrence of the detectors applied to the characterisation of EOs using MDGC

the ^{2}D column, to perform proper qualitative and quantitative characterisation.

Detection Systems

The detectors utilised in the past 10 years have mostly been either flame ionization detectors (FID), qMS, or TOFMS (Fig. 8; Tables 1, 2). For qualitative analysis qMS and TOFMS are the detectors of choice. FID is often coupled with qMS since it offers higher acquisition frequency, thereby improving the accuracy of 2D peak areas used for quantitative purposes [64]. Applied in multiplex systems, FID has also been shown to give more repeatable results, compared to qMS alone, probably again due to the same reason [57]. TOFMS, with its high data acquisition frequency across a broader mass range, spectral deconvolution of overlapping peaks, and selectivity, is a widely used hyphenated detector with MDGC [4, 17]. Von Muhlen et al. [43] showed that TOFMS had better peak resolution and definition, compared to qMS, with a higher signal-to-noise ratio, with TOFMS identifying 15% more compounds than qMS, which is important for the analysis of complex matrices such as EOs. TOFMS showed another advantage over qMS, in that it gives a higher similarity with the MS library than qMS. The explanation given for this was that TOFMS was less affected by mass spectral distortion than qMS. Also, the higher acquisition frequency needed to perform GC×GC is easier to obtain using TOFMS [43].

An advantage of the triple quadrupole mass spectrometer (QqQMS) is the option to monitor the presence and abundance of certain specific ions in the system, using multiple reaction monitoring (MRM) and selected ion monitoring (SIM) operational modes. SIM mode can be very useful for the identification and quantification of trace-level compounds co-eluting with more abundant ions. Also, since there are many compounds coming from the same chemical class, the application of MRM mode can be a useful tool in monitoring the levels of compounds containing the same functional group (targeted analysis), as well as in the trace analysis. These operational modes offer specific, selective, sensitive, and quick characterisation of target peaks, managing also to perform untargeted analysis of EOs [66] and increasing the identification capabilities of the MDGC system. The disadvantage of QqQMS is that spectral similarity between experimentally obtained mass spectra and library

spectra is lower compared to qMS. This comes from the fact that MS library, FFNSC 2.0 used in Tranchida study [66], was built with GC/qMS systems, which can differ significantly from QqQMS. MSMS systems coupled with GC × GC can analyse the high peak density produced by GC × GC, which cannot be followed up by switching of ion selection in the quadrupole section. This can be overcome using cold-electron ionization instead of hard-electron ionization [96]. Also, there is a decrease in spectral similarity observed for GC × GC/MSMS, compared to GC/MSMS, due to the differences in the flow of effluent entering ion source (e.g. 8.8 mL min^{-1} vs. 1.3 mL min^{-1}, respectively), which is the case in flow modulated GC × GC. A similar observation was made for GC × GC/qMS and GC/qMS [66].

With a high acquisition range of 15–1500 amu, with ability to provide peak purity, and perform real-time calibration, HR-TOFMS is able to provide mass accuracy of ± 0.002 amu. These features enable a HR-TOFMS system to provide enhanced mass spectral data sets for chromatograms obtained in MDGC, enabling deconvolution of co-eluting compounds [36].

Chemometric Techniques in MDGC Optimisation

Besides their application in data analysis, chemometric techniques can also be employed in the optimisation of MDGC processes. Omar et al. [63] applied experimental design to optimise the GC × GC parameters. Central Composite Design (CCD) and Multisimplex were utilised to optimise several parameters, including modulation period, discharge time, first column flow, and second column flow, using the peak volume of each analyte as the response, which is directly related to sensitivity and resolution [63]. A full review of the application of chemometric techniques to MDGC-generated data will be provided in Part 2 [97].

Novel MDGC Approaches

To increase the resolution of EO separation, many authors have tried to take simultaneous advantage of both heart-cut and comprehensive modes. This principle has been developed in two different ways. The first is that the effluent from the ^{1}D column can be split in two ^{2}D columns, one long, and a second short column. All compounds are transferred to both ^{2}D columns, without separating and targeting certain regions and compounds [77]. The second principle is that several selected regions are focused with cryotraps and transfered, using a Deans Switch to a long column (GC–GC), while the other regions are sent to a short column (GC × GC). Since the ^{2}D column dimensions are different, even though both have the same stationary phase, there is a difference in retention times and resolution. Furthermore, the application of the Deans Switch can influence retention times, giving a time lag for the compounds separated in the heart-cuts. Application of this system on lavender EO showed good reproducibility, while the use of a cryotrap enabled an increase in peak heights due to the refocusing of the re-injected fraction [52].

Sasamoto et al. [83] developed a 1D/2D GC/MS system using a CFT Deans Switch, and a dual low thermal mass GC, to perform a quick and easy switch between one-dimensional and two-dimensional systems without any change in instrumental setup (see Fig. 9). One of the options of this system was to perform a preliminary FID analysis to select specific heart-cuts for further and more in-depth analysis, as well as

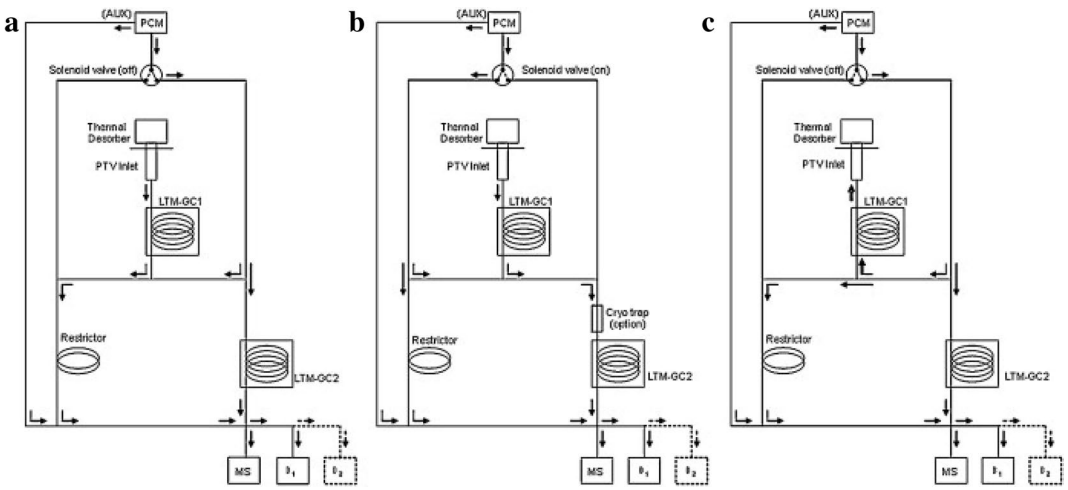

Fig. 9 Schematic flow diagrams for a selectable 1D/2D GC/MS system; **a** 1D GC/MS; **b** heart-cutting; **c** 2D GC/MS analysis 1D GC back flush; D_1: selective element-specific detector; D_2: selective element-specific detector. Reproduced with permission from [83]

monitoring the total ion chromatogram for the ^{1}D column during the run with the GC–GC system.

Multiplexed Dual Column Comprehensive Systems

GC × GC is state-of-the-art technology for the analysis of EOs, and using short and narrow-bore columns in the second dimension, separation can occur in a few seconds. However, when using thermal modulation, a flow mismatch can occur between the two dimensions due to the column serial configuration. Also, since the column in ^{2}D is short and narrow, with a thinner stationary phase, in most cases having same phase ratio, it can easily be overloaded. This decreases the resolution on the ^{2}D column, particularly where minor peaks present are in close proximity to more abundant compounds and thus co-elute. The solution is to build a multiplex system which has two parallel ^{2}D columns (GC × 2GC). The other advantage of a dual-column system in the ^{2}D is the synergism of dual detectors. MS is known to provide a fundamental contribution to analyte identification, while FID offers a continuous data collection, thereby improving the accuracy of 2D peak areas used for quantitative purposes. This can be observed in the example of the analysis of the EO obtained from *A. umbelliformis* Lam. Two of the most abundant compounds in this EO are α- and β-thujone which, in the case of standard GC × GC co-elute with the minor compounds, nonanal and 2-methylbutyl isovalerate. By applying a GC × 2GC system, these two minor compounds were separated and identified.

The second great advantage of this system is that it provides consistent results, in terms of analyte characterisation, offering internal cross-validation of quantification and accuracy [64]. Nicolotti et al. reported upon the characterisation of a GC × 2GC system, performed through validation parameters such as linearity, precision, and accuracy against a reference standard. Using a loop-type thermal modulator, cryotrapping and refocusing of the sample at the head of the ^{2}D was achieved, offering higher loading capacity with an increase in peak capacity, resolution, and overall orthogonality. Results for the most common authenticity markers for *Mentha* spp., with an absolute quantitation error smaller than 13%, were reported as being in agreement or even better than the standard [8]. Such results can be achieved as GC × 2GC offers better chromatographic resolution, which leads to better separation and easier quantification of desired compounds [57].

In comparison to the multiplex system with the two columns in the second dimension, Savareear et al. [65] set up a system with two columns with different stationary phase polarities in the first dimension (see Fig. 10). This was possible because of a contra-directional modulation approach, according to which two ^{1}D columns were aligned so neither one of them blocked the jet of cool gas onto the other column. The injected sample splits into two ^{1}D columns and later recombines into one stream on the ^{2}D column. As a result, two 2D contour plots are obtained containing complementary information. The great advantage of this approach was that different interactions in the first dimension between solutes and stationary phases lead to different contributions in the ^{2}D column. One of the disadvantages of this system was that quantitation of the analytes needed to be performed by normalization of peak areas, as the split between the two columns in ^{1}D was not necessarily always even.

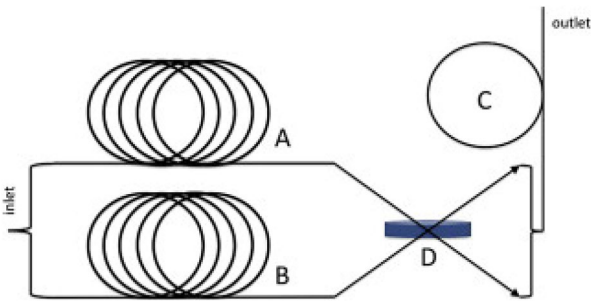

Fig. 10 Scheme of a multiplex 2GC × GC system **a** ^{1}D Column 1: PEG; **b** ^{1}D Column 2: dimethyl polysiloxane; **c** ^{2}D column: (50%) phenyl (50%) dimethylpolysiloxane; **d** dual-stage cryogenic modulator. Reproduced with permission from [65]

Preparative MDGC

Besides application in the characterisation of complex samples, as a separation technique MDGC can be applied to the isolation and purification of compounds of interest from complex mixtures. 1D GC has already been established as a preparative method [98]. However, the low peak capacity of one column leads to significant co-elution and comparatively low purity of the isolated compound. To increase the amount of the purified compound in preparative gas chromatography, samples large enough are injected to overload the column, which significantly decreases the separation power of the system. This is a big problem for 1D GC system because the purity of the final product is even lower. However, overloading is necessary to increase the amount of isolated compound and decrease total run time. Thus, megabore columns, with 0.53 mm inner diameter and 0.5–1.0 µm coating, which can manage high sample volumes, are widely used in preparative chromatography.

The use of additional columns, as in MDGC, with different stationary phases providing some degree of orthogonality, allows isolation of pure compounds while at the same time decreasing overloading effects [99–101]. Thus, in recent years, MDGC has increased in importance for preparative purposes.

Eyres et al. [99, 100] developed microscale preparative GC–GC by trapping compounds eluting from the ^{1}D column with a LMCS and applying a microfluidic Deans Switch to selectively isolate the compound of interest eluting from the outlet of the ^{2}D column, instead of transferring it to a ^{2}D detector. By doing 100 injections they managed to isolate 77.6 µg of geraniol with 75.5% recovery from a mixture of peppermint, spearmint, and lavender EOs with a purity of 99.3%. The amount obtained was enough for nuclear magnetic resonance (NMR) spectroscopy analysis. For the isolation of 1,4-dimethoxybenzene from peppermint EO using the same system, the recovery, with cryotrap set on − 20 °C, was 52.2%. Decreasing the temperature of the cryotrap, from − 20 to − 25 °C and − 30 °C, led to recoveries of 63.4% and 76.8%, respectively, showing the importance of cryotrap optimisation. The same system was applied by Ruhle et al. [102] separating pairs of compounds from a mixture of peppermint, spearmint and lavender EOs and obtaining them with high purity. However, separated carvone and linalyl acetate showed lower purity due to the overlapping peaks and overloading of the GC–GC system. Better recovery without the presence of interfering peaks can be achieved with more precise oven settings in the critical target region.

Compared to previous preparative 2D GC systems with cryotraps and Deans switches, Sciarrone et al. [101] applied a three-dimensional heart-cut GC (GC–GC–GC) preparative system, equipped with three Deans Switch devices to enable efficient consecutive heart-cuts, where after each injection the isolated compound was collected with a low-cost lab-constructed collection system. Sciarrone et al. used three mega-bore columns with the thickest non-polar column coating in ^{1}D, 5 µm, guaranteeing the greatest sample capacity, while in ^{2}D and ^{3}D the column coating thicknesses were 2.0 and 0.8 µm. The inner diameter and coating of the columns applied by Sciarrone are thicker compared to those applied by Eyres. This enabled the application of three times higher sample volumes than in microscale preparative GC, obtaining at the same time higher amounts of isolated compounds. After three consecutive heart-cut steps, the purity of carotol was 99.3%, which was much better than 1D preparative GC, where carotol was co-eluting with three more interfering compounds, decreasing its purity to 70%.

Preparative GC–GC can be applied to determine the structure of unknown compounds as well as to confirm their structure via ^{1}H NMR analysis. This way it is possible to identify compounds for which a match cannot be found in a MS library [99, 100]. Chin et al. [78] applied a peak enrichment technique to separate a compound from an EO. This technique provides on-line and in situ enrichment of the compounds that are heart-cut using a microfluidic Deans Switch device, cryotrapped, and stored after each injection. The main reason for developing this technique was to isolate and detect compounds that were present in trace amounts, or have a low *S/N* ratio, or for detectors that require a larger injected mass of compounds to provide adequate identification [78]. This technique can be also applied for the isolation of compounds for their quantification or for use as reference standards. This is of importance, because many compounds present in EOs cannot be found as commercial pure reference standards. Such was the case with α-amorphene and β-vetivone, for which standards could not be found, so Sciarrone et al. [103] isolated them from Haitian vetiver EO applying a GC–GC–GC system. The addition of LC to a GC–GC system, was used as a pre-separation step with the aim of providing class-separation of sesquiterpenes and oxygenated sesquiterpenoids, allowing introduction of 5–10 times higher amounts of samples, due to the increased sample capacity of LC compared to GC.

Panto et al. applied [104] 3D LC–GC–GC and GC–GC–GC systems (Fig. 11) for the isolation of seven highly pure sesquiterpenoid alcohols from sandalwood EO. With a suitable choice of column configuration and

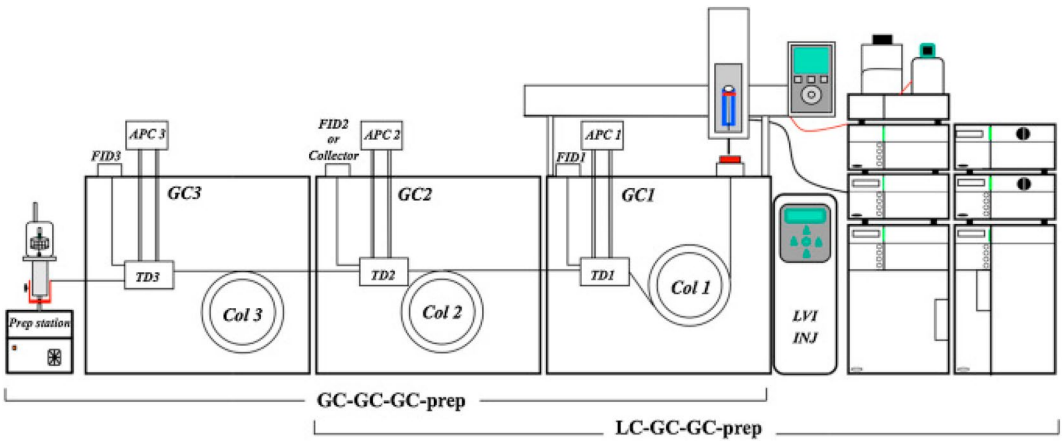

Fig. 11 Scheme of 3D LC–GC–GC and GC–GC–GC; the two configurations used are marked. Reproduced with permission from [104]

heart-cuts, compounds co-eluting in the ^{1}D were purified and collected individually. Application of LC, with all its aforementioned advantages when coupled with a GC–GC system, was exploited for the purification of low abundance compounds such as α-bisabolol, (*Z*)-lanceol, and (*Z*)-nuciferol [104]. Sciarrone et al. [105] showed that the application of an appropriate column configuration can lead to separation of one compound in each dimension with high levels of purity. By setting (5%) diphenyl (95%) dimethylsiloxane as the ^{1}D column, patchouli alcohol was isolated, while after a ^{2}D (100%) polyethylene glycol column α-bulnesene was isolated, and after a custom-made ionic liquid-based ^{3}D column, α-guaiene was purified from the patchouli EO. In this way, in only one process, three compounds were isolated, each collected individually without any interfering compounds.

Concluding Remarks and Future Perspectives

The work performed in the past years shows that MDGC clearly has higher separation power when compared to conventional GC. This is especially observed in the analysis of complex matrices, such as EOs. In EO analysis, when untargeted separation is preferable, GC × GC has a clear advantage over the heart-cut technique. However, in targeted analysis, such as the enantioselective analysis of EOs, when only a few pairs of compounds need to be characterised, GC–GC has the advantage because of the longer ^{2}D column, which provides higher peak capacity compared to the short column in GC × GC. Novel approaches in MDGC take the advantage of both the heart-cut and comprehensive modes, combining them into one system. In addition, several multiplex systems have been developed to increase the power of separation on the ^{2}D column, decreasing the loaded effluent from the ^{1}D column to the ^{2}D and obtaining two different separation profiles when two different columns were applied in ^{1}D. This clearly shows that in the future even more information about the EOs can be obtained from the chromatographic process, helping provide faster and more accurate peak identification, EO authentication, and quality assurance.

In addition to technological advances, increased performance of MDGC can be obtained by optimisation of existing systems. By introducing an off-line pre-separation step, the complexity of certain EOs can be decreased, which leads to increased resolution in subsequent MDGC analysis. This can be applied in EO characterisation and also for preparative purposes. In the past years, only LC was used as a pre-separation step in EOs analysis. In the future, it can be expected that other extraction techniques will be connected with the MDGC system to determine some compounds present at trace levels, such as pesticides, allergens, sensitizers, etc. As already established, for the most efficient separations, the stationary phases in the two dimensions must have different selectivity, while the dimensions of the columns must be optimised according to the complexity of the EO. Fast separations can be performed in a system containing a shorter column, and hyphenated with advanced MS systems such as QqQ or HR-TOFMS, which can perform both targeted and untargeted qualitative and quantitative characterisation and spectral deconvolution of the overlapped peaks. Thus, application of high-performance detectors can improve the quality of chromatograms, and increase characterisation capabilities of the whole MDGC system in EO analysis. In addition, modulation period has a great impact on the quality of separation and must be very well optimised. Due to the complexity of the optimisation process in MDGC, in the future it is expected that experimental design will become more important in the optimisation process.

Acknowledgements Authors would like to thank University of Tasmania, Australia and Plant Therapy Inc., Twin Falls, Idaho, USA, for their generous provision of a jointly funded PhD scholarship and project support for Leo Lebanov.

Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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