Journal of Chromatography A, 1217 (2010) 1522–1529

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Development of a switchable multidimensional/comprehensive two-dimensional gas chromatographic analytical system

Bussayarat Maikhunthod, Paul D. Morrison, Darryl M. Small, Philip J. Marriott*

Australian Centre for Research on Separation Science, School of Applied Sciences, RMIT University, G.P.O. Box 2476, Melbourne 3001, Australia

ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form
23 December 2009
Accepted 24 December 2009
Available online 4 January 2010

Keywords:
Comprehensive two-dimensional gas chromatography
GC × GC
Multidimensional gas chromatography
Deans switch
Aroma-impact
Lavender oil

ABSTRACT

In this study, a new system for analysis using a dual comprehensive two-dimensional gas chromatography/targeted multidimensional gas chromatography (switchable GC × GC/targeted MDGC) analysis was developed. The configuration of this system not only permits the independent operation of GC, GC × GC and targeted MDGC analyses in separate analyses, but also allows the mode to be switched from GC × GC to targeted MDGC any number of times through a single analysis. By incorporating a Deans switch microfluidics transfer module prior to a cryotrapping device, the flow stream from the first dimension column can be directed to either one of two second dimension columns in a classical heart-cutting operation. Both second columns pass through the cryotrap to allow solute bands to be focused and then rapidly remobilized to the respective second columns. A short second column enables GC × GC operation, whilst a longer column is used for targeted MDGC. Validation of the system was performed using a standard mixture of compounds relevant to essential oil analysis, and then using compounds present at different abundances in lavender essential oil. Reproducibility of retention times and peak area responses demonstrated that there was negligible variation in the system over the course of multiple heart-cuts, and proved the reliable operation of the system. An application of the system to lavender oil, as a more complex sample, was carried out to affirm system feasibility, and demonstrate the ability of the system to target multiple components in the oil. The system was proposed to be useful for study of aroma-impact compounds where GC × GC can be incorporated with MDGC to permit precise identification of aroma-active compounds, where heart-cut multidimensional GC-olfactometry detection (MDGC-O) is a more appropriate technology for odour assessment.

1. Introduction

The development of advanced instrumentation and techniques for flavour and aroma-impact compound investigation is ongoing. The general aim is to achieve improved separation power and identification capabilities [1]. Importantly, flavour or aroma-impact compounds give unique odour characteristics for particular products, such as e.g. fragrances, food and beverages. There is continuing interest in the flavour industry to analyse odourant compounds in products. Gas chromatography (GC) is a basic technique applied in a range of aroma compound research [2]. For further identification of compounds, data obtained from GC-FID may be supplemented with various spectroscopic detectors, e.g. Fourier transform infrared, quadrupole (qMS) and ion-trap mass spectrometry, and off-line NMR [3]. In addition, where the target compounds contribute to the odour quality, GC coupled with organoleptic detection using the human nose has been applied to characterise aroma-impact. However, the basic problems of one-dimensional GC (1DGC) separation, including co-elution and trace presence of analytes, still occur and remain a difficulty in compound identification [4,5].

The multidimensional gas chromatography (MDGC) technique plays an important role in the area of flavour studies owing to its enhanced separation capability. There are two primary means by which the MDGC technique is applied, i.e. comprehensive two-dimensional gas chromatography (GC × GC) and classical MDGC where discrete heart-cut fractions are transferred from a first dimension column to a second, on which improved separation is sought. GC × GC has been claimed to offer many advantages over 1DGC. It has been demonstrated to have excellent retention time reproducibility, and very high peak capacity. It also is claimed to provide enhanced sensitivity due to zone compression, allowing the determination of trace analytes that may not be detectable by 1DGC. The principles and diverse applications of GC × GC have been described elsewhere [6–9]. Data generation and presentation as a contour plot can be usefully employed in chemical profiling or mapping of a sample's constituents, in ways not possible by 1DGC, allowing comparison amongst different sources of material such as herbs, environmental samples, petroleum products and so

* Corresponding author. Tel.: +61 3 99252632; fax: +61 3 99253747.
E-mail address: philip.marriott@rmit.edu.au (P.J. Marriott).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.12.078

forth. Identification of separated compounds from GC × GC is readily achieved by suitable coupling with a high data acquisition rate mass detector such as time-of-flight mass spectrometry (TOFMS) or fast quadrupole MS (qMS). Di et al. [10] analysed Chinese herbal mixtures for various alkaloids (ephedrines, etc.) and reported chiral separation of these components in various tonics. The study of hop oil extracts for flavour analysis was undertaken by Eyres et al. who interpreted data obtained from GC-olfactometry (GC-O) and that from GC × GC–TOFMS and finally resorted to MDGC-O in order to gain more precise identification of aroma-impact compounds [11,12]. Despite increased peak capacity over 1DGC, GC × GC analysis was unable to definitively identify the target spicy aroma compounds, consequently unambiguous assignment of target compounds in complex samples is not always possible, especially for data correlated with GC-O. To address this problem, d'Acampora Zellner et al. [13] proposed an alternative technique by coupling a sniff port to the GC × GC system. However, the modulation of GC × GC and generation of multiple peaks to produce narrow peaks (100–400 ms) are generally too short for the typical breathing cycle of humans (3–4 s) and makes this approach impractical [5]. The targeted MDGC approach can be an alternative way to deal with this issue.

MDGC target analysis improves the separation of discrete selected regions from a first dimension (^{1}D) separation. Only the target region will be heart-cut and transferred for further separation on the second dimension (^{2}D). By contrast, in GC × GC the whole sample is continually applied to separation through ^{2}D. Transfer of analyte in MDGC is best accompanied by a cryotrapping step, to reduce dispersion of the transferred band and effectively allow a very narrow band to be introduced to the ^{2}D column. This permits narrow and fast elution conditions to be used, and ensures minimum broadening at the injection step. Marriott et al. [14] reported a novel approach to MDGC, wherein a directly coupled column set comprising a first long column and a shorter fast elution column with a moving cryomodulator is located near the column junction. Thus no switching or other interface was required. Subsequently Marriott et al. [15] developed a heart-cutting process that delivered similar performance. The process involved holding the cryotrapping unit, which envelopes the capillary column segment, in position for an extended time to completely trap a target compound, then moving the cryotrap towards the inflowing carrier stream direction to permit rapid mobilization of the band to the second column. A relatively short ^{2}D column of about 5 m was used. This process may be repeated any number of times. Dunn et al. [16] applied the targeted MDGC technique to quantification of co-eluting peaks of suspected allergens in fragrance products. Begnaud and Chaintreau [17] applied a similar process that was based on a loop cryotrapping column modulator segment arrangement with MDGC-O to chiral separations, to evaluate the odour intensity, and description of enantiomers. Eyres et al. [18] proposed the study of an aroma-impact compound in essential oils by comparing data from a sequence of GC-O, GC × GC-FID and MDGC-O analyses. In the final analysis only MDGC was able to adequately separate the odour compound to permit it to be tentatively identified and quantified, with the target aroma-impact cluster from GC × GC analysis now well resolved by MDGC.

The study of flavour compounds still requires the combination of data obtained from an organoleptic detector and physical detectors in order to gain more reliable interpretation. The capacity of GC × GC as a sensitive technique with enriched separation data serves a valuable role in this area. However, improved identification of aroma-impact character by using MDGC-O also is desired. Up till now, flavour studies require separate experiments for implementation of each of these techniques, with subsequent correlation of data [4,11,12,18,19]. Therefore, the present investigation aims to develop a new separation system by inclusion of these two elegant separation techniques, i.e. targeted MDGC and GC × GC, in one unified system which we have termed switchable MDGC/GC × GC operation. The idea is to serve the requirement for significantly better separation and precise aroma-impact characterisation of targeted regions, whilst still gaining an overview of the total sample composition from GC × GC separation, now in one analytical system. Method validation used a series of essential oil mixtures, and then was applied to a more complex sample (here, lavender) in order to affirm its capability. The proposed system aims to be a model platform for future study to provide integration with other applications, and also detectors. Thus the MDGC-O emphasis here arises from the detection speed that requires slower olfactory sampling, however other applications that require better peak capacity than that offered by the second dimension in GC × GC could be usefully studied.

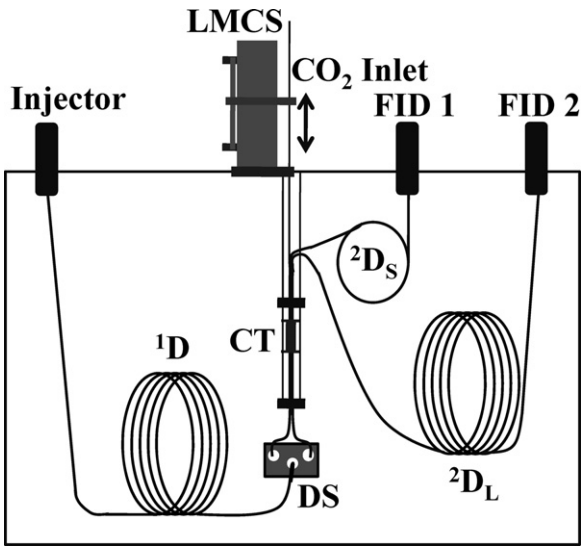

Fig. 1. Schematic diagram of the switchable targeted MDGC/GC GC × GC system. DS: Deans switch; CT: cryotrap; ^{1}D: first dimension column; ^2D_S: short second dimension column (for GC × GC mode) terminated at Flame Ionization detector FID 1; ^2D_L: long second dimension column (for targeted MDGC mode) terminated at Flame Ionization detector FID 2.

2. Experimental

2.1. Materials

The following standard essential oil components were provided by Australian Botanical Products (Hallam, Australia. Stated purity in parentheses); γ-terpinene (98.98%), mixture of menthone and iso-menthone (97.08%; containing 80.08% menthone and 17% iso-menthone), geraniol (98.93%), limonene (96.95%), linalool (97.09%), geranyl acetate (93.52%), bornyl acetate (100%), linalyl acetate (97.02%), neryl acetate (97.29%), and Bulgarian lavender oil (92.23%). 1-Octanol was purchased from Ajax Finechem (NSW, Australia); acetone (<99.0%) was purchased from Merck (VIC, Australia); and ethanol (99.5%) was purchased from Ajax Finechem (NSW, Australia).

2.2. Gas chromatographic system configuration

All analyses were carried out using a newly developed system described further below (see Fig. 1) using an Agilent 6890A gas chromatograph (Agilent Technologies, Little Falls, DE, USA) equipped with a model 7683 Series auto-sampler and dual FID detectors (FID 1; FID 2). The instrument was retrofitted with an Everest model Longitudinally Modulated Cryogenic System (LMCS;

Chromatography Concepts, Doncaster, Australia). A cryogenic trapping (CT) segment was placed at the beginning of the secondary columns, one of which was long and of regular ID, and the other was short and of narrow bore. The basic operation of the LMCS has been described elsewhere [15,20]. The column set consisted of two different phase capillary columns. A 30 m × 0.25 mm I.D. × 0.25 μm film thickness (d_f) BPX5 (5% phenyl polysilphenylene–siloxane stationary phase, SGE International, Australia) primary column (^{1}D) coupled in series to dual parallel secondary columns, one of short length (^2D_S) and the other longer (^2D_L). Each was connected in turn to individual FID detectors. The ^2D_L column was 30 m × 0.25 mm I.D. × 0.25 μm d_f BP20 (polyethylene glycol stationary phase, SGE International) on which MDGC separation was performed. The ^2D_S was a 0.786 m × 0.1 mm I.D. × 0.1 μm d_f BP20 column on which the GC × GC separation was performed.

An Agilent Deans switch interface (DS; part number G2855B), which functions as a microfluidics sample transfer device, was located between the end of the ^{1}D column and the beginning of the ^{2}D columns. By use of the Deans switch, the eluate from the ^{1}D column can be selectively directed to either: (i) FID 1 via the ^2D_S column with GC × GC operation or (ii) FID 2 via the ^2D_L column with targeted multidimensional GC operation. The design of the Deans switch interface, principle of operation and application to various studies are published elsewhere [21,22]. In some cases cryotrapping is not implemented and the overall process is simply one of transferring solute directly to either the ^2D_S or ^2D_L columns. In order to provide switching flow, additional carrier gas was supplied to the Deans switch using a three-channel auxiliary electronic pressure control (EPC) module (G1570A, Agilent Technologies). The valve switching operations and cryogenic trap movement were controlled through the Chemstation events control.

In an initial experiment, Agilent Deans switch calculator software (version A.01.01, Agilent Technologies) allowed balance of the flows to permit effective complete transfer of the primary column flow to either secondary channel. The inlet and auxiliary EPC pressures were then fine-tuned using methane, and geraniol injections under isothermal oven temperature conditions. This ensures that pneumatic switching is efficiently performed such that 100% of ^{1}D eluate is sent to either the ^2D_S or ^2D_L column. A constant inlet pressure of 16.6 psi (114.1 kPa) and a constant auxiliary pressure of 15.0 psi (103.4 kPa) provided a suitable balanced set point and were used for the remainder of the study.

2.3. System validation

A standard mixture, consisting of γ-terpinene, octanol, menthone (+iso-menthone), geraniol, geranyl acetate, and bornyl acetate, was prepared at a concentration of 100 mg L^{-1} in acetone and used to validate the system. The following conditions were applied: the oven temperature was programmed from 60 to 220 °C at 5 °C min^{-1}. Sample injections of 1 μL were conducted with an injector temperature of 220 °C with split ratio of 10:1. Hydrogen was used as a carrier gas with the constant inlet and auxiliary pressures as stated above. Both detectors were operated at 230 °C with acquisition rates of 20 Hz, except when GC × GC mode was implemented, when the FID 1 acquisition rate was 100 Hz. The system validation experiments were conducted as follows.

2.3.1. Conventional GC separation ^{1}D–^{2}D

The standard mix was separated on ^{1}D followed by transfer through (a) ^2D_S to FID 1 or (b) ^2D_L to FID 2. In this instance, the cryomodulator cooling was not engaged. This allows contrast of the FID response magnitude through each arrangement, for each compound, with each detector.

2.3.2. Switching efficiency of selected regions

Initially, the standard mix was separated on ^{1}D and directed to the ^2D_S column. The conventional analysis above (Section 2.3.1a) serves as a reference chromatogram. During the analysis the Deans switch valve was programmed to transfer 3 selected discrete regions from ^{1}D (targeted regions: γ-terpienene, menthone (+iso-menthone), bornyl acetate) to ^2D_L for further separation. The other three compounds (octanol, geraniol, geranyl acetate) were directed through the ^2D_S column. The timing of the switching events was set according to the start and end elution times of the respective three target peaks through the ^2D_S column as recorded for the reference data in Section 2.3.1a for FID 1. This appraises the effectiveness of the Deans switching valve during an analysis, to ensure there is complete transfer, and also to determine whether there are changes to retention times arising from the switching valve action. The retention times of the three selected compounds on FID 1 and their peak areas were compared with those obtained from Section 2.3.1a.

2.3.3. Heart-cutting efficiency with cryotrapping

The experiment described in Section 2.3.2 was repeated, but now with cryotrapping of the heart-cut components at the start of the ^2D_L column. This permits the heart-cut regions to be separately focused before being remobilized into the ^2D_L column. The efficiency and the peak area and height of individual peaks were compared with those observed from the operation described in Sections 2.3.1b and 2.3.2.

2.3.4. GC × GC separation

This operation investigates the effectiveness of the configuration in terms of GC × GC separation, to ensure suitable modulation action of the cryotrapping device. The standard mix was separated on ^{1}D followed by direct transfer to the ^2D_S column. The modulation period (P_M) was set at 5 s and the cryogenic trap was maintained at −20 °C for the duration of analysis.

2.3.5. GC × GC/targeted MDGC operation

This operation incorporated two operations in one analysis; both GC × GC and targeted MDGC. Three compounds (octanol, geraniol and geranyl acetate) were analysed by GC × GC through the ^2D_S column using the same condition as Section 2.3.4. During this time three targeted regions (γ-terpinene, menthone (+iso-menthone) and bornyl acetate) were analysed by targeted MDGC operation through the ^2D_L column. The selected compounds for each respective mode were determined by timing of the flow path of the Deans switch. In addition, the mode of LMCS movement has to be conducted according to the specific operation which was performed. The modulation control was manually switched to continual modulation (M) mode for GC × GC operation (e.g. a fixed P_M setting of 5 s), to focus and re-mobilize compounds to the ^2D_S column. Modulation control was then manually switched to target (T) mode when MDGC operation was required. In this operation, the modulator is held for a sufficient duration to collect the complete component(s) then by moving the modulator the targeted region is released rapidly to the ^2D_L column. The required duration of target peak collection (modulator hold time) was predicted from the elution period of each peak obtained from normal GC mode through ^2D_S (Section 2.3.1a). Thus in this operation the LMCS was manually switched between M- and T-mode during the analysis. The timing of switching between the two modes has to be integrated with the modulation period to ensure that the target operation is an integer value of the P_M setting, so that the modulation timing will remain unchanged throughout the operation. Synchronisation is provided by ensuring that the target mode reverts to GC × GC operation in a time that is an integer value of the modulation period. Thus the duration of the target zone should be a multiple of an integral num-

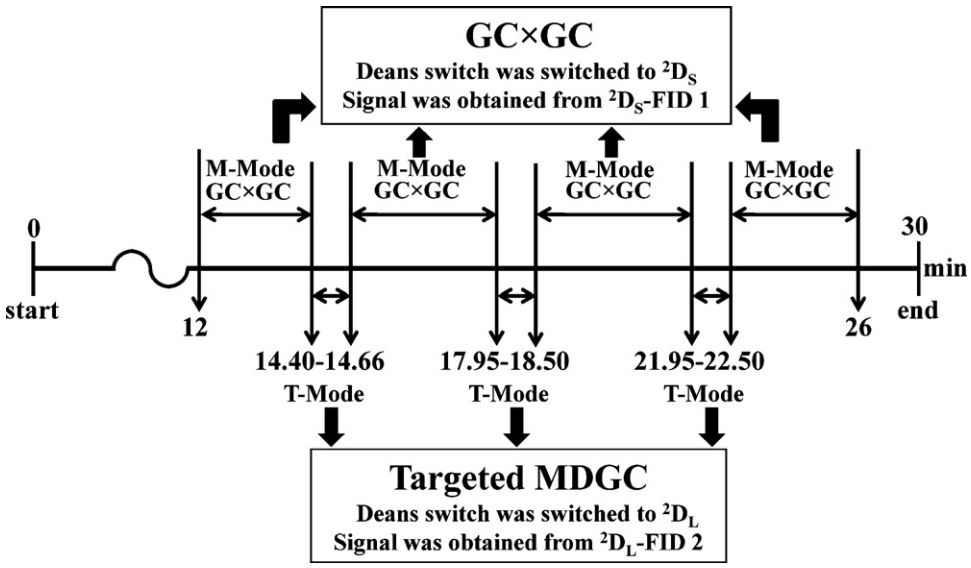

Fig. 2. Events chart for switching operation between GC × GC and targeted MDGC separation modes according to Section 2.3.5.

ber of the modulation period (although this is not essential). This will ensure that exactly the same retention, separation pattern and resolution of compounds will be attained, compared to the analysis where GC × GC operation is conducted for the whole analysis. Also, it should be able to perform precise heart-cutting processes for targeted MDGC separation. A typical events chart for the switching operation for standard mixture analysis is shown in Fig. 2, where three target zones (T-mode) are switched to the ^2D_L column whilst the other regions are operated under modulation conditions (M-mode). For GC × GC operation in the present case, the contour plot can be directly compared with the contour plot from that in Section 2.3.4 (and peak positions should overlap in these two plots). Additionally, the positions and responses of peaks from MDGC operation may also be compared with the result from Section 2.3.3.

2.4. Application of the system to lavender oil

Lavender oil (50,000 mg L^{-1} in ethanol) was used as a more complex test sample. A reference mixture, consisting of limonene, linalool, linalyl acetate, bornyl acetate and neryl acetate, was prepared at a concentration of 100 mg L^{-1} in ethanol. Lavender oil sample and the reference mixture were separately analysed through a series of experiments similar to that carried out in Section 2.3. The reference mixture provided the appropriate target regions for heart-cutting of these compounds from the lavender oil sample. Therefore, for GC × GC/targeted MDGC analysis, after separation on ^1D, here only these 5 heart-cut regions were separated by targeted MDGC operation through the ^2D_L column; the remainder of the analytes were separated by GC × GC operation through the ^2D_S column. The GC condition was the same as applied in the validation step, except an oven temperature programming rate of 3 °C min^{-1} was employed. An injection volume of 0.2 μL of lavender oil sample was made. The modulation period was set at 6 s for GC × GC separation. The heart-cut durations for targeted MDGC separation were 16.50–16.95, 19.95–20.45, 27.30–28.12, 29.62–30.10, and 33.00–33.65 min, respectively. The results from each operation were compared, and will be described below.

3. Results and discussion

3.1. System validation

Since the proposed system development is to establish a system which can perform two separation techniques, the dual GC × GC and targeted MDGC, in one analysis run, the initial validation will investigate systematic steps to demonstrate performance of each test experiment. Meanwhile, such a system should be potentially also capable of operation in a single mode (i.e. conventional GC, GC × GC or targeted MDGC operation) to serve each of these requirements as necessary. Therefore, system validation was performed across these several procedures in order to demonstrate and confirm the reliable functioning of the system.

According to the column configuration (Fig. 1), the two ^2D columns were different in their dimension, but comprise the same stationary phase (BP20, polyethylene glycol). Thus the responses, in terms of retention time and resolution, are expected to be differ-

Table 1
Area ratio[a] of each compound in the standard mixture obtained from each operation during system validation, standard deviation (SD) determined from four repeat analyses.

Compound	Average area ratio (SD)				
	Experiment				
	^2D_S-FID 1 [Section 2.3.1a]	^2D_L-FID 2 [Section 2.3.1b]	^2D_L-FID 2 [Section 2.3.2]	^2D_L-FID 2 [Section 2.3.3]	^2D_L-FID 2 [Section 2.3.5]
γ-Terpinene	0.94 (0.09)	0.92 (0.08)	0.93 (0.09)	0.94 (0.09)	0.91 (0.06)
Octanol	0.87 (0.09)	0.88 (0.09)			
Menthone	0.59 (0.06)	0.60 (0.06)	0.60 (0.06)	0.59 (0.06)	0.62 (0.05)
iso-Menthone	0.21 (0.02)	0.21 (0.02)	0.22 (0.02)	0.21 (0.02)	0.22 (0.02)
Geraniol	0.85 (0.08)	0.88 (0.09)			
Bornyl acetate	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Geranyl acetate	0.81 (0.04)	0.83 (0.04)			

[a] Area ratio is a ratio between peak area of the compound *versus* the bornyl acetate peak. This is done to account for injection volume variations.

Table 2
Retention times (min) of each compound in the standard mixture obtained from each operation during the system validation, standard deviation (SD) determined from four repeat analyses.

Compound	Average retention time (SD)				
	Experiment				
	^2D_S-FID 1 [Section 2.3.1a]	^2D_L-FID 2 [Section 2.3.1b]	^2D_L-FID 2 [Section 2.3.2]	^2D_L-FID 2 [Section 2.3.3]	^2D_L-FID 2 [Section 2.3.5]
γ-Terpinene	14.553 (0.002)	15.784 (0.004)	15.789 (0.004)	15.895 (0.000)	15.895 (0.001)
Octanol	14.772 (0.001)	16.727 (0.002)			
Menthone	18.033 (0.003)	19.539 (0.004)	19.551 (0.004)	19.984 (0.000)	19.984 (0.000)
iso-Menthone	18.362 (0.003)	19.910 (0.004)	19.923 (0.006)	20.046 (0.000)	20.047 (0.000)
Geraniol	20.747 (0.003)	23.012 (0.005)			
Bornyl acetate	22.047 (0.004)	23.520 (0.004)	23.533 (0.003)	24.051 (0.000)	23.957 (0.000)
Geranyl acetate	24.623 (0.004)	26.182 (0.005)			

ent even though the separations were carried out under the single operational condition. Peak areas will depend on the optimised conditions of each FID detector. However, regardless of the different column dimensions, the ratio of FID response amongst the compounds is expected to remain closely similar. The area ratio between peak areas of each compound in the standard mixture *versus* the bornyl acetate peak obtained from each operation mode is illustrated in Table 1, with the retention times of compounds given in Table 2. Without engagement of cryomodulator cooling, the area ratio obtained from $^1D/^2D_S$-FID 1 was not significantly different to those obtained from $^1D/^2D_L$-FID 2. This can be interpreted as there being little variation of injection quantity and good transfer of solute through either the ^2D_S or ^2D_L column; it also suggests the FID responses are well matched. However, the functional difference in retention times observed from $^1D/^2D_S$-FID 1 is that they are earlier than those from $^1D/^2D_L$-FID 2, due to a much shorter column length of ^2D_S.

The period of cryotrapping for each compound was defined according to the elution time from conventional GC by times on $^1D/^2D_S$-FID 1. When the Deans switch valve operation was implemented during an analysis by transferring three target regions for further separation through the ^2D_L column (Section 2.3.2), the switching events had negligible effect on responses. This is illustrated by the similar area ratio and the remarkably close retention times of compounds to those observed from the conventional GC by $^1D/^2D_L$-FID 2 separation (Section 2.3.1b; Table 2). Thus for γ-terpinene direct operation with solute passed from ^1D to ^2D_L gave 15.784 min, but heart-cutting with switching to ^1D–^2D_L gave 15.789 min—a difference of only 0.005 min. The largest difference noted (e.g. for bornyl acetate) with values of 23.520 and 23.533 min, was 0.013 min–0.8 s. This was after 3 such Deans switching events. The retention times of target compounds that arise from the switching action appear to be less than 0.02 min, compared with conventional GC separation. This also confirms the favourable functioning of the Deans switching valve. This is reflected in the high similarity of the responses in Fig. 3A and B. In order to evaluate the heart-cutting efficiency with cryotrapping (Section 2.3.3), the same three target regions were cryo-collected before release for further separation through the ^2D_L column. The area ratio of each compound was still similar to the heart-cutting operation in the absence of the cryogenic fluid.

As expected, the retention times from this operation are delayed owing to the cryotrapping step. In comparison, γ-terpinene showed the least delay of retention time, most probably due to the shorter period of cryotrapping time (14.40–14.66 min). Menthone and iso-menthone were cryotrapped for a longer period in order to completely trap both compounds within this period of time (17.95–18.50 min). Bornyl acetate was also cryotrapped for a longer period (21.95–22.50 min) due to the apparent tailing of the peak, which also resulted in a delayed retention time. In terms of peak shape and width reduction, a substantially increased peak height of around 7-fold was found; see Fig. 3C. As a general observation, the peak heights increased, whilst the peak width was reduced compared to Fig. 3B. However, the comparison of increased detection sensitivity in terms of signal-to-noise ratio (S/N) in Table 3 showed the S/N to increase by about 3–4-fold once cryotrapping was applied. The impact of cryofocussing in terms of the increase in peak height of targeted peaks, and the decrease in the width at half height by a factor 2–7, leads to an improved sensitivity as described by Marriott et al. [14].

For validation of the switching operation between GC × GC and targeted MDGC modes, the retention times and area ratio of three heart-cut compounds were also compared with the previous oper-

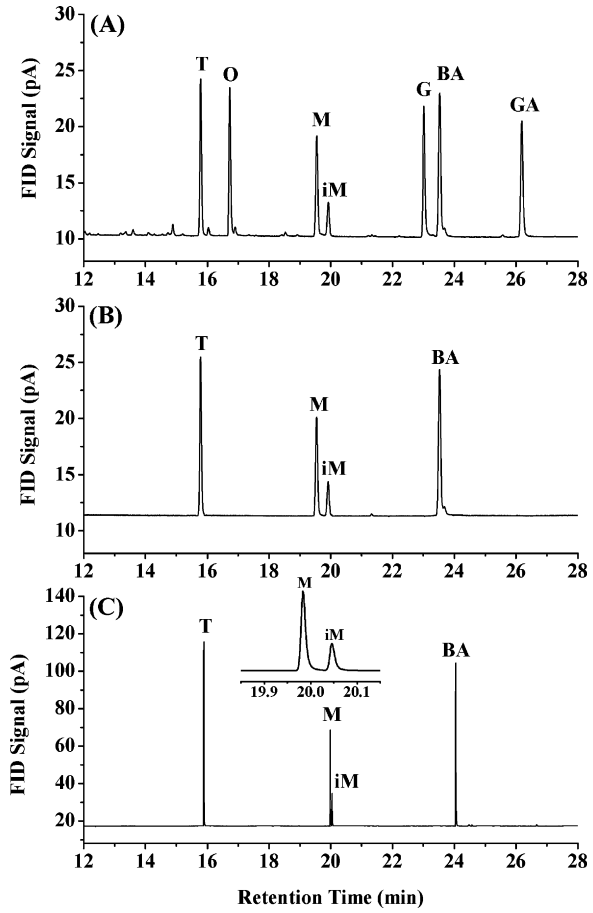

Fig. 3. Chromatograms of the standard mixture separated via (A) $^1D/^2D_L$-FID 2; (B) $^1D/^2D_S$, with Deans switch heart-cutting to ^2D_L-FID 2; (C) Operation as in (B) but now cryotrapping of each heart-cut zone is carried out. [T: γ-terpinene; O: octanol; M: menthone; iM: iso-menthone; G: geraniol; BA: bornyl acetate; GA: geranyl acetate]. Inset is an expanded chromatogram of menthone and iso-menthone. Refer to Table 2 for respective retention times of the components in the different operations. Peaks in (C) are delayed due to the cryotrapping operation.

Table 3
Signal-to-noise ratio (S/N) of each compound in the standard mixture obtained from each operation during the system validation, standard deviation (SD) determined from four repeat analyses.

Compound	S/N (SD)				
	Experiment				
	^2D_S-FID 1 [Section 2.3.1a]	^2D_L-FID 2 [Section 2.3.1b]	^2D_L-FID 2 [Section 2.3.2]	^2D_L-FID 2 [Section 2.3.3]	^2D_L-FID 2 [Section 2.3.5]
γ-Terpinene	67.7 (2.9)	66.9 (3.3)	275.0 (27.7)	1115.1 (58.0)	1573.6 (174.0)
Octanol	62.7 (4.2)	68.1 (4.9)			
Menthone	37.8 (2.5)	39.6 (2.8)	159.5 (21.7)	520.4 (24.1)	845.4 (99.0)
iso-Menthone	12.9 (0.9)	13.3 (0.9)	54.8 (7.2)	177.6 (6.6)	285.3 (31.9)
Geraniol	50.7 (2.6)	57.3 (3.5)			
Bornyl acetate	56.8 (2.9)	58.1 (2.3)	239.4 (26.5)	914.8 (121.8)	1457.6 (82.5)
Geranyl acetate	45.6 (1.5)	48.0 (1.1)			

ations. Area ratios closely matched those of the other operations. The retention times obtained from this method showed them to be nicely reproduced with the targeted MDGC result (Table 2). In addition, a comparison of the contour plot from this dual operation with an entire GC × GC analysis (Section 2.3.4) is demonstrated in Fig. 4. Note that the event chart in Fig. 2 refers to the operation given in Fig. 4. The vertical lines in Fig. 4A are the heart-cut regions for GC × GC switched to targeted MDGC operation, where these compounds were directed away from GC × GC-FID 1 to the other channel, and they do not appear on the FID 1 signal. Hence they do not appear in Fig. 4B. A separate ^1D–^2D_L FID 2 signal is not shown here since essentially the same result as in Fig. 3C is attained. The contour plot (B) reveals that heart-cutting and targeted MDGC operation can perform a nice clean and precise transfer of eluate. Although the elution times of compounds separated by GC × GC operation were shifted marginally, the same pattern of separation was obtained. The ^2t_R of octanol is 1.0 s and 1.1 s in Fig. 4A and Fig. 4B, respectively; that of geraniol is 1.2 s in Fig. 4A and 0.9 s in Fig. 4B; and that of GA is 1.0 s in Fig. 4A and 0.7 s in Fig. 4B. This observation will be considered below. From the above results, it can be stated that the operation of LMCS by switching between target (T)- and modulation (M)-mode during an analysis could be well performed, and the programmed timing events for the switching of separation between GC × GC and targeted MDGC operations was successful.

The maximum length of the ^2D_L column section that can be used will depend upon the column polarity, the compound polarity, the phase, and peak retention factor, to normally ensure a previous target region is fully eluted from the ^2D_L column before the next target region is introduced. This will determine how closely two neighboring target zones can be selected and passed into the ^2D_L column. It is possible that such zones might be less than the $^2t_{Rmax}$ on the column, since a subsequent target zone could be delivered to the column provided the two zones do not overlap on ^2D_L.

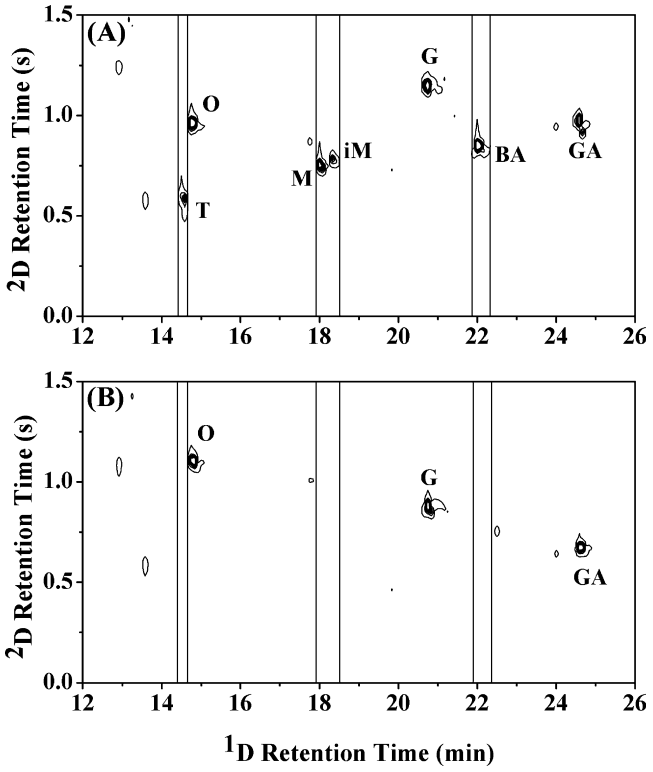

Fig. 4. Successive comprehensive two-dimensional gas chromatography results of the standard mixture. (A) GC × GC operation for the entire analysis according to Section 2.3.4. (B) Switching operation GC × GC and targeted MDGC separation according to Section 2.3.5, with the ^2D_S-FID 1 result shown. Vertical lines in (A) correspond to the heart-cut regions selected for switching to the ^2D_L column and hence are not presented in the FID 1 detector response in (B). A P_M value of 5 s was used, but only a 1.5 s expansion is shown. The separate ^2D_L-FID 2 result is not shown here. For abbreviations, see Fig. 3.

Table 4
Peak area, area ratio[a] [in square parentheses] and peak height of five compounds in lavender oil separated via ^2D_L-FID 2 by using two operations. Standard deviation (SD) determined from four repeat analyses.

Compound	Average area (SD)		Average peak height (SD)	
	Experiment			
	Conventional GC	GC × GC/targeted MDGC (heart-cut regions)	Conventional GC	GC × GC/targeted MDGC (heart-cut regions)
Limonene	92.1 (1.56)/[12.45(0.030]	93.4 (1.36)/[12.11 (0.53)]	18.9 (0.21)	121.6 (3.56)
Linalool	666.4 (15.63)/[90.04 (0.039)]	668.6 (22.44)/[86.65 (2.130]	128.2 (3.18)	406.2 (18.85)
Linalyl acetate	655.9 (7.92)/[88.64 (0.620]	663.5 (21.33)/[85.00 (2.30)]	99.0 (1.91)	634.9 (28.12)
Bornyl acetate	3.1 (0.14)/[0.42 (0.01)]	3.8 (0.21)/[0.49 (0.01)]	0.7 (0.02)	3.7 (0.16)
Neryl acetate	7.4 (0.14)/[1.00 (0.00)]	7.7 (0.45)/[1.00 (0.00)]	1.4 (0.07)	7.1 (0.38)

[a] Area ratio is a ratio between peak area of the compound *versus* the neryl acetate peak.

3.2. Application of the system to lavender oil

In order to prove the system feasibility for application to a complex sample, lavender oil was used as a sample test. The reference mixture was analysed to obtain the retention time data of the peaks as shown in Fig. 5A. For the GC × GC/targeted MDGC operation, these five identified targeted regions of lavender oil sample were then heart-cut after ^{1}D separation and sent through the ^2D_L column for further separation. The data records of peak area, area ratio against the bornyl acetate peak response and peak height were subsequently compared with data from conventional GC via ^2D_L-FID 2, as shown in Table 4. The peak area of each compound indicates excellent reproducibility which is similar to the finding from the validation study. In addition, the peak height increased significantly when cryotrapping was applied. The contour plots were also compared between the plots from an entire GC × GC separation (Fig. 6A) and GC × GC/targeted MDGC separation (Fig. 6B), where the target peaks were cut to the ^2D_L column, and so are absent from the FID 1 signal. In this example, both abundant (LL, LA, LM) and trace (NA, BA) components were selected for this test.

Although lavender oil is a complex sample, the heart-cutting process can still be carried out precisely according to programmed timing events to transfer the required target peaks. Generally, although the small shifting of retention time on the second dimension can be observed (not more than 0.1 s) the separation pattern and resolution remain essentially the same. As illustrated in Fig. 6C, the chromatogram from targeted MDGC of the 5 heart-cut regions reveals a good separation as well. Note the BA and NA peaks shown in Fig. 5B were now transferred to the ^2D_L column, cryotrapped and eluted on this column, and gave the result in Fig. 6C in the expanded trace. The comparison of the responses of these components with the result in Fig 5B is clear, and thus the relative responses of NA and B in Fig. 5B are the same as in Fig. 6C.

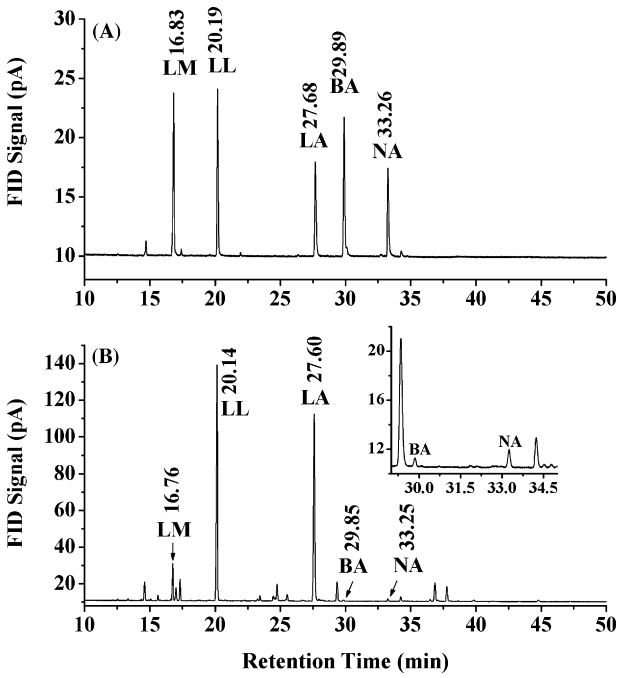

Fig. 5. Chromatograms of (A) the reference mixture and (B) the lavender oil sample, retention times of each targeted peak are shown. Inset is an expanded chromatogram of bornyl acetate and neryl acetate. These chromatograms are separated via the 1D/^2D_S-FID 1 system, with cryotrapping not employed [LM: limonene; LL: linalool; LA: linalyl acetate; BA: bornyl acetate; NA: neryl acetate].

Close inspection of Figs. 4 and 6 reveals that some peak positions are not exactly reproduced in the GC × GC trace operated under Section 2.3.4 (GC × GC) and Section 2.3.5 (GC × GC/MDGC). This is believed to be due to the method for integrating the GC × GC opera-

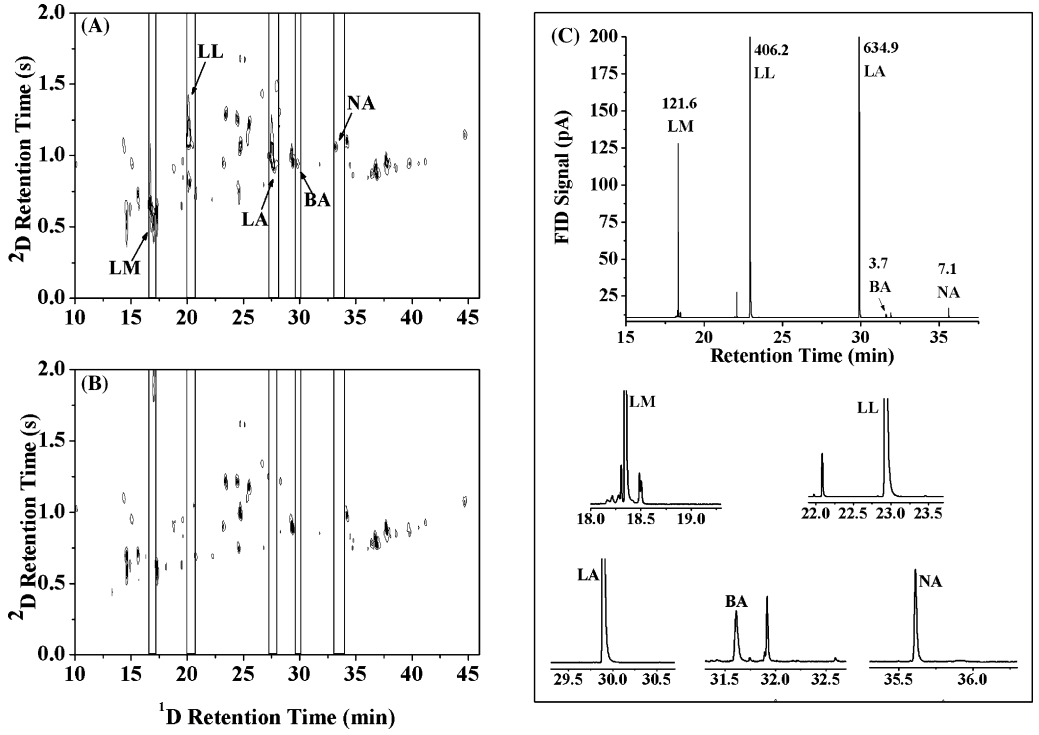

Fig. 6. Results from lavender oil analysis. (A) Contour plot obtained from GC × GC separation, (B) Contour plot obtained from GC × GC/targeted MDGC separation, Vertical lines correspond to the regions where the Deans switch is activated, so the compounds in this chromatogram are absent since they have been cut to MDGC operation. (C) Chromatogram of the five heart-cut regions with peak height from targeted MDGC separation shown. Expanded MDGC chromatograms are shown at the lower part of panel (C). A P_M value of 6 s was used, but only a 2 s expansion is shown. For abbreviations, see Fig. 5.

tion with MDGC. In Fig. 4, 5 s modulation is used, which is a period of 0.08333 min. The LMCS controller is able to provide precise timing under 5 s modulation, however when target mode is implemented this operation reverts to Chemstation software control, to halt the modulation process for the required duration. When modulation control is then recommenced, Chemstation must instruct the modulator to operate under its own timing at a given time in the event control. Since there is a limitation in the precision of setting the Chemstation events times (only two decimal places is permitted) then setting of modulation recommencement time (which should be in multiples of 0.08333 min) is only allowed a precision of 2 decimal places (i.e. the entry is rounded to 0.08 min) in the events table. The peaks appear to be shifted by the error produced in excessive rounding of the timing control. This can be of the order of 0.18 s. For 6 s modulation (Fig. 6) the setting is much more precise, since 0.01 min can be correctly set in the Chemstation events table to recommence modulation, and the peak positions are now well reproduced. It should be noted that this type of operation cannot be anticipated as being required for an events table for software in GC–normally precision to 0.01 min would be satisfactory for any event, provided it is able to set this time accurately and reproducibly. Thus, care should still be exercised when setting the switching time events between T- and M-mode for LMCS movement; it is important to ensure the timing for target operation will not alter the modulation period consistency and commencement of each modulation event for GC × GC operation, or at least have as little variation as possible. As a consequence, the pattern of GC × GC separation should remain unchanged throughout an analysis, and this is largely shown to be true for the data reported in Fig. 6A and B.

The idea of the combination of GC × GC and targeted MDGC operations to be able to select any desired region and so to potentially obtain better resolved peaks for a region of co-eluting peaks from the ^{1}D column, by permitting selection of targeted MDGC separation on a longer ^{2}D column has a number of desirable outcomes. It allows a full mapping of all compounds as a contour plot by using GC × GC separation in a first analysis, and then to select any subsequent region for target analysis. Where, for instance, a region was to be selected for olfactory analysis, which requires a longer duration for sniffing of the effluent from the column, as was reported in earlier work [18], the present strategy should accommodate this study. Previous studies on fast enantioseparation of chiral compounds [23] reported the difficulty of achieving chiral separation in the second column of a GC × GC separation, on the time scale required to elute compounds under GC × GC conditions. The described system with a longer enantioselective column operated in MDGC mode would seem a possible compromise. From the above results, it can be stated this newly developed system can potentially serve such a requirement. Note that the arrangement as presented is a proof of concept that has shown the various capabilities of the integrated system to be possible. It is now necessary to test the individual performances of each dimension to evaluate if they meet the requirements of optimum or best possible performance. This will be a continuing study.

4. Conclusions

The system described above should serve as a model for any system where an overview GC × GC analysis is required for a sample, but where limited peak capacity of the ^{2}D column in GC × GC is insufficient to adequately resolve some components from a sample mixture. An example of this is proposed where olfactory analysis of components in a sample demands slower sensory perception of individual peaks, and makes GC × GC technology incompatible with GC-O analysis. Since the aim of GC × GC analysis is to provide a more complete separation of components than can be achieved with 1DGC, for regions of chromatograms that exhibit too many overlapping peaks in GC × GC, this method can offer an appropriate solution by switching that region to a longer column. Likewise, application to enantioselective analysis would appear to make this a suitable technology for chiral analysis.

Acknowledgments

Dr John Fergeus of Australian Botanical Products is gratefully thanked for kind provision of the standard compounds used in this study. B.M. gratefully thanks the Royal Thai Government for a Ph.D. scholarship.

References

[1] P.J. Marriott, G.T. Eyres, J.-P. DuFour, J. Agric. Food Chem. 57 (2009) 9962.
[2] L. Mondello, A.C. Lewis, K.D. Bartle, Multidimensional Chromatography, John Wiley & Sons, Chichester, 2002.
[3] G.T. Eyres, S. Urban, P.D. Morrison, J.-P. Dufour, P.J. Marriott, Anal. Chem. 80 (2008) 6293.
[4] M. Parker, A.P. Pollnitz, D. Cozzolino, I.L. Francis, M.J. Herderich, J. Agric. Food Chem. 55 (2007) 5948.
[5] C.M. Delahunty, G. Eyres, J.-P. Dufour, J. Sep. Sci. 29 (2006) 2107.
[6] T. Gorecki, J. Harynuk, O. Panic, J. Sep. Sci. 27 (2004) 359.
[7] P. Marriott, R. Shellie, Trends Anal. Chem. 21 (2002) 573.
[8] T. Gorecki, O. Panic, N. Oldridge, J. Liq, Chromatogr. Relat. Technol. 29 (2006) 1077.
[9] R. Shellie, P. Marriott, A. Chaintreau, Flavour Fragr. J. 19 (2004) 91.
[10] X. Di, R.A. Shellie, P.J. Marriott, C.W. Huie, J. Sep. Sci. 27 (2004) 451.
[11] G. Eyres, J.-P. Dufour, G. Hallifax, S. Sotheeswaran, P.J. Marriott, J. Sep. Sci. 28 (2005) 1061.
[12] G.T. Eyres, P.J. Marriott, J.-P. Dufour, J. Agric. Food Chem. 55 (2007) 6252.
[13] B. d'Acampora Zellner, A. Casilli, P. Dugo, G. Dugo, L. Mondello, J. Chromatogr. A 1141 (2007) 279.
[14] P.J. Marriott, R.C.Y. Ong, R.M. Kinghorn, P.D. Morrison, J. Chromatogr. A 892 (2000) 15.
[15] P. Marriott, M. Dunn, R. Shellie, P. Morrison, Anal. Chem. 75 (2003) 5532.
[16] M.S. Dunn, N. Vulic, R.A. Shellie, S. Whitehead, P. Morrison, P.J. Marriott, J. Chromatogr. A 1130 (2006) 122.
[17] F. Begnaud, A. Chaintreau, J. Chromatogr. A 1071 (2005) 13.
[18] G. Eyres, P.J. Marriott, J.-P. Dufour, J. Chromatogr. A 1150 (2007) 70.
[19] S. Rochat, J. Egger, A. Chaintreau, J. Chromatogr. A 1216 (2009) 6424.
[20] P.J. Marriott, R.M. Kinghorn, Anal. Chem. 69 (1997) 2582.
[21] D.R. Deans, Chromatographia 1 (1968) 18.
[22] J. Blomberg, U.A.T. Brinkman, J. Chromatogr. A 831 (1999) 257.
[23] R. Shellie, P.J. Marriott, Anal. Chem. 74 (2002) 5426.