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Conventional and narrow bore short capillary columns with cyclodextrin derivatives as chiral selectors to speed-up enantioselective gas chromatography and enantioselective gas chromatography mass spectrometry analyses

Carlo Bicchi, Erica Liberto, Cecilia Cagliero, Chiara Cordero, Barbara Sgorbini, Patrizia Rubiolo*
Dipartimento di Scienza e Tecnologia del Farmaco - Università degli Studi di Torino
Via Pietro Giuria, 9 10125 Torino – Italia

Address for correspondence:
Prof. Dr. Patrizia Rubiolo
Dipartimento di Scienza e Tecnologia del Farmaco, Facoltà di Farmacia,
Università degli Studi di Torino, Via Pietro Giuria 9, Turin 10125, Italy;
Fax: +39 011 6707687; e-mail: patrizia.rubiolo@unito.it

Abstract
The analysis of complex real-world samples of vegetable origin requires rapid and accurate routine methods, enabling laboratories to increase sample throughput and productivity while reducing analysis costs. This study examines shortening enantioselective GC (ES-GC) analyses following the approaches used in fast-GC. ES-GC separations are conditioned by a weak enantiomer-CD host-guest interaction and the separation is thermodynamically driven and strongly influenced by temperature. As a consequence, fast temperature rates can interfere with enantiomeric discrimination; thus the use of short and/or narrow bore columns is a possible approach to speeding-up ES-GC analyses. The performance of ES-GC with a conventional inner diameter (I.D.) column (25 m length x 0.25 mm I.D., 0.15 μm and 0.25 μm d_f) coated with 30% of 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin in PS-086 is compared to those of conventional I.D. short column (5 m length x 0.25 mm I.D., 0.15 μm d_f) and of different length narrow bore columns (1, 2, 5 and 10 m long x 0.10 mm I.D., 0.10 μm d_f) in analysing pesticide standard, racemate standards and real-world-samples in the flavour and fragrance field.
Short conventional I.D. columns gave shorter analysis time and comparable or lower resolutions with the racemate standards, depending mainly on analyte volatility.
Narrow-bore columns were tested under different analysis conditions; they provided shorter analysis time and resolutions comparable to those of conventional I.D. ES columns. The narrow-bore columns offering the most effective compromise between separation efficiency and analysis time are the 5 and 2 m columns; in combination with mass spectrometry as detector, applied to lavender and bergamot essential oil analyses, these reduced analysis time by a factor of at least three while separation of chiral markers remained unaltered.

Keywords: fast enantioselective GC-MS, cyclodextrin derivatives, linear retention indices, mass spectra, flavours, fragrances.

1. Introduction

Cyclodextrin derivatives (CDs) have gained an increasing acceptance as chiral stationary phases (CSPs) in GC separation of enantiomers because of their wide enantioselectivity and ability to separate underivatized enantiomers of different volatilities. When used in enantioselective gas chromatography (ES-GC), CDs are generally diluted in apolar or moderately polar polysiloxanes to obtain highly efficient capillary GC columns, as proposed by Schurig *et al.* [1-3]. The success of a separation technique depends on its suitability for use in the routine analysis of real-world samples, whose requirements include the ability to identify characteristic markers in a sample and short analysis time. In general, a correct enantiomeric excess (EE) or enantiomeric ratio (ER) determination of optically active markers in complex real-world samples requires a two-dimensional approach, since enantioselective analysis can make the resulting chromatographic profile more complex, because enantiomer separation may cause peak doubling, increasing the risk of peak overlap. Two complementary but distinct approaches are those most commonly adopted: the first and most popular one is based on a second dimension *in separation* with heart-cut GC-GC [4-7] or comprehensive two-dimensional GC (GCxGC) [8-11]; the second approach includes a second dimension *in identification* using mass spectrometry (MS) (or very rarely FT-IR) as ES-GC detector. MS is known not to be a selective chiral probe making enantiomer MS spectra indistinguishable. These spectra can therefore successfully be exploited to determine EE or ER correctly in both the total and the extract ion modes although they can only be used to locate the enantiomers in the chromatogram since their linear retention indices (I^T) [12] are necessary to identify them unequivocally [13].

One of the approaches used to satisfy the increasing demand for analytical controls is to adopt fast chromatographic techniques, which enable high sample throughput and laboratory productivity, while at the same time reducing analysis costs. fast-GC and fast-GC-MS have long been established for routine analysis [14-19]; these methods involve contemporary contributions of

several parameters including column length and inner diameter (I.D.), stationary phase film thickness (d_f), temperature programme and linear carrier gas velocity. A limit of ES-GC in routine quality control is the long analysis time since the separation depends on a small difference in the energy of the association between each enantiomer and the CD chiral selector and requires a very high chromatographic efficiency. The ES-GC separation of enantiomers by CD derivatives is known to be based on fast kinetics and entirely governed by thermodynamics [20, 21], and therefore strongly controlled by temperature. In contrast to what happens with fast conventional GC, the temperature rates that can be used to speed up an ES-GC separation with CDs as CSPs are therefore rather low. Fast-ES-GC can thus mainly be achieved by operating on column length, inner diameter and/or flow-rate and in particular with short narrow bore (NB) columns. These columns not only increase analysis speed and analyte detectability due to peak sharpening [22], but also reduce enantiomer elution temperature, resulting in a gain of enantioselectivity that compensates (in full or in part) for the loss of efficiency (N). Short columns were applied in ES-GC with CDs since the beginning of the 1990s enabling enantiomer separations even in a few seconds [23-26]. After an in-depth study of ES-GC, Schurig and Czesla [27] concluded that short conventional 0.25 mm I.D. columns should be used for fast-ES-GC because of their good loadability, integration characteristic, use of conventional instrumentation and lower consumption of carrier gas.

However, conventional I.D. short columns can be applied in monodimensional ES-GC only when optically active compounds must be determined in low complexity samples and/or when a low number of enantiomers must be analysed simultaneously. With medium-to-high complexity samples, a highly-efficient separation system combined with single- or multiple-ion monitoring-MS detection is necessary to determine EE and/or ER correctly. This article examines how to optimise the parameters conditioning the speeding up of ES-GC-MS analysis of medium-to-high- and medium-to-low-volatility analytes; it also looks at the application of fast-ES-GC-qMS to routine analysis of real world samples in the field of flavours and fragrances.

2. Experimental

2.1 Samples

Pure standards of racemic linalool, linalyl propionate, γ-lactones (C6, C7, C8, C11, C12, C14, C15), δ-octalactone, α–HCH, trichlorfon, *trans*-chlordane, and heptachlor, together with the components of the laboratory-prepared chiral test mixture consisting of limonene, 2-octanol, camphor, isobornyl acetate, linalyl acetate, 2-methyl-(3Z)-hexenyl butyrate, menthol, hydroxycitronellal, γ-decalactone and δ-decalactone racemates [28], were from the collection of standards in the authors' laboratory or, if unavailable there, were obtained from Sigma–Aldrich (Milan, Italy). All standard compounds were dissolved in cyclohexane at a concentration of 100 ppm each. Solvents were all HPLC grade from Riedel-de Haen (Seelze, Germany). Lavender (*Lavandula angustifolia* P. Mill.) and bergamot (*Citrus bergamia* Risso et Poiteau) essential oils (e.o.), obtained by hydrodistillation following the method described in the European Pharmacopoeia (6th edition) [29]**,** were diluted 1:200 in cyclohexane before analysis.

2.2 Instrumental set-up

A Shimadzu QP2010 GC-MS system provided with Shimadzu GCMS Solution 2.51 software (Shimadzu, Milan, Italy) was used.

Columns: GC analyses were carried out on the set of columns reported in table 1 and coated with 2,3-di-O-ethyl-6-O-*tert*-butyldimethylsilyl-β-CD (2,3DE6TBDMS-β-CD) [30] as CSP diluted at 30 % in PS086. All columns were from MEGA (Legnano, Italy).

GC-MS conditions: injection mode: split; split ratio: 1: 20 for conventional I.D. column and split ratio 1:100 for narrow bore (NB) columns; injection volume: 1µl. Temperatures: injector: 220°C, transfer line: 230°C; ion source: 200°C; carrier gas: He. The MS was operated in electron impact ionization mode (EI) at 70 eV, the scan rate was 1111 u/s and a mass range of 35–350 m/z suitable to cover the full fragmentation pattern of most e.o. components was chosen. Flavour and fragrance

compounds were analysed starting from 50°C while pesticides from 100°C. Temperature programmes and flow rates are listed in table 1. Resolution was calculated with the following equation: $R= 1.18 (t_{R(2)} - t_{R(1)})/(w_{0.5(1)} + w_{0.5(2)})$

3. Results and discussion

This section is divided into two main parts: the first comprises an evaluation of the effect of temperature and flow rates, stationary phase film thickness, inner diameter and column length on speeding up and performance of ES-GC or ES-GC-MSwith CD derivatives as CSPs, and the second concerns the application of these techniques to the analysis of real-world samples in the e.o. field. Some pesticide standard racemates were also analysed to extend the range of structures and characteristics of the analytes investigated. If not specified otherwise, all results are compared to those obtained with a 25 m long, 0.25 mm I.D., 0.25 μm d_f taken as reference column (hereafter called "reference column").

3.1 Effect of column length, inner diameter, film thickness and chromatographic conditions on the speed of an enantioselective GC analysis.

One of the conditions for a correct ES-GC analysis is that the enantiomers of the chiral compounds investigated are baseline separated must to afford a correct EE (or ER) determination. The excess of efficiency with several chiral compounds of the columns coated with the last generation of CD derivatives can be exploited to shorten analysis time by acting on temperature and flow rates, stationary phase film thickness, inner diameter and column length. This approach has here first been applied to the analysis of a group of standards of racemates with different polarity and volatility (table 2) with a set of columns coated with 2,3-DE-6TBDMS-β-CD (30% in PS-086) as CSP [30] that includes the reference column, two conventional 0.25 mm I.D. columns 5 m long x 0.15 μm d_f and 25 m x 0.15 μm and four 0.10 mm narrow bore (NB) columns 1, 2, 5, and 10 m long and 0.10 μm d_f. The influence of the different columns and analysis conditions on the separation performance of the chromatographic systems was evaluated by the separation measure, *S*, [31], calculated by analysing a chiral test mixture used routinely in the authors' laboratory [28]. The adopted analysis conditions were chosen to achieve the simultaneous baseline separation of as many chiral components as possible for a given real-world sample. Analyses were therefore operated in temperature programmed mode and not in isothermal mode, as it is usual for theoretical studies where the separation of each chiral analyte must be optimized.

The first experiments concerned the influence of length and film thickness on the performance of conventional I.D. columns. All experiments were run at a heating rate of 2 °C/min, without optimising elution temperatures. Table 2 reports retention time (t_R) of the second eluted enantiomer, elution temperature of both enantiomers (T_{el}1-2), resolution (R) and % analysis time reduction calculated on the second eluting enantiomer of the chiral standard compounds investigated, when analysed with conventional I.D. columns of different lengths (25 and 5 m) and film thicknesses (0.25 and 0.15 μm). Figure 1 reports the ES-GC separation of α-HCH with the three 0.25 I.D. columns in question. The results show that a thinner CSP film thickness produces a different reduction of analysis time, ranging from 37% for limonene to 13% for γ-pentadecalactone, with increased resolution for all analytes. These results can be explained by the decreased elution temperature, –between 13°C –and 20°C lower, which improves the separation capability of the chromatographic system.

Under the same conditions, the effect of column length is even more evident. Compared to the 25 m - 0.15 μm d_f, analysis times with the 5 m - 0.15 μm d_f column decreased from about 69% for limonene to about 26% for γ-pentadecalactone, with a loss of resolution ranging from about 65% for γ-heptalactone to about 10% for heptachlor. As expected, the decreased efficiency due to column shortening had a bigger influence on the resolution of the better-separated enantiomers. Moreover, the percent reduction of analysis time decreased with analyte volatility, as shown with

the homologous series of γ-lactones, where it ranges from about -60% with γ-hexalactone to -26% with γ-pentadecalactone. The combined effect of thinner CSP film thickness and column shortening produced a reduction in analysis time ranging from about 80% for limonene to 35% for γ-pentadecalactone, with a decrease in elution temperature ranging between 28°C and 55°C. In real terms, the limonene analysis time decreased from about 17 to 3.5 min, from about 69 to 44 min for γ-pentadecalactone and from about 28 to 6 min for α-HCH; for the last two analytes , the resolution remained almost unaltered.

Column length strongly influences the separation capability of the ES-GC system: the separation measure *S* calculated on the chiral test mixture for the reference column decreased by more than 50% with the 5 m x 0.15 μm d_f column (from 942 to 491) while S was comparable (i.e. 972) when film thickness was reduced to 0.15 μm with the 25 m column (table 3).

This loss of separation capability (and efficiency) of the chromatographic system can affect routine analysis of real-world samples, but can be overcome by increasing efficiency with columns with narrower I.D. [14-19] and/or selectivity by applying MS detection in extract ion mode. With the same phase ratios, narrow-bore columns are known to give higher efficiency compared to conventional I.D. columns, thus enabling shorter columns to be used. The most widely used I.D. is 0.10 mm, because the resulting columns enable reasonable split ratios and/or dilutions that avoid column overloading, while reducing analysis time by a factor varying from 4 to 10. The same value of enantiomer resolution of 1.5 was adopted as the limit of analysis acceptance. Two racemates with different volatility and polarity (linalool and γ-pentadecalactone) and the chiral test mixture [28] were analysed with four 0.10 mm I.D. columns of different lengths (10, 5, 2, 1 m) but coated with the same CSP film thickness (0.10 μm of 30% 2,3-DE-6TBDMS-β-CD in PS-086) and under different temperature and flow rates. All analysis were started at the same temperature (50°C). The ES-GC conditions are reported in table 1. Figure 2 shows the ES-GC patterns of linalool analysed with the four NB columns under investigation, at 1 mL flow rate at 10°C/min. Figure 3 shows the relationships of resolution (A) and retention time (B) *versus* flow and temperature rates of γ-pentadecalactone. In the diagram A, the limit of accepted resolution (1.5) is shown as a full line. The highest resolution with linalool was obtained in all cases with the 5 m narrow bore column, followed by that 2 m long. The low values for the 10 m column are not surprising and may be due to the long linalool residence time in the column, which increases elution temperature. Linalool resolution in all conditions was above 1.5; thus the 1 m column could be used successfully, with a reduction in the analysis time of about 95% compared to the reference column (24.3 min *vs.* 1.1 min) and of 80% compared to the 10 m NB column (5.3 min *vs.* 1.1 min) when quicker ES-GC conditions are applied. Similar considerations can be made for γ-pentadecalactone, for which the 5 and the 2 m NB columns provided base-line separation up to 5°C/min in contrast to the 10 and 1m NB columns, with which 1.5 resolution could only be achieved at 2°C/min. With the 5 and 2 m NB columns, the reduction in analysis time under the quickest conditions (1.0 mL/min – 5°C/min) was about 65% (69.1 min *vs.* 24.7 min) compared to the reference column and 56% compared to the 10 m NB column base-line separation (56.8 min *vs.* 24.7 min).

The most effective NB columns are therefore the 2 m and the 5 m long columns, the latter probably offering the best compromise between analyte residence time and system separation efficiency. These considerations were confirmed when the separation capability of the four NB columns was measured on the chiral test mixture through the separation measure *S*. Table 3 clearly shows that *S* for the 5 m NB column is higher than that of 10 m column and more than double the value for the corresponding conventional I.D. 5m column, even at 10°C/min. The low separation capability of the 10 and 1 m NB columns is due to different factors: the efficiency of the 1 m NB column is too low and is not compensated for by temperature decrease, while for the 10 m NB column, efficiency is high but the high elution temperature interferes with effective chiral discrimination

The reliability of fast ES-GC-MS with NB columns *versus* EE and ER determination was also evaluated, by analysing linalool with the four NB columns investigated under all applied analysis

conditions. Table 4 shows % areas of each enantiomer on the total areas of the *R*- and *S*-linalool enantiomers and related SD and RSD% compared to those obtained with the reference column

3.2 Fast ES-GC/MS analysis of real-world samples with narrow bore columns

The second part of this section deals with the analysis of real-world samples. Two commercially important essential oils of medium complexity, i.e. lavender and bergamot, were analysed by ES-GC-MS.

Lavender essential oil contains several optically active components: α- and β-pinene, camphene, β-phellandrene, limonene, 1-octen-3-ol, camphor, linalool, borneol, linalyl acetate, terpinen-4-ol, lavandulol, α-terpineol and lavandulyl acetate. A sample of lavender essential oil was first analysed with the four NB column at different temperature rates (2, 3.5, 5 and 10°C/min) and at constant He flow of 1 mL/min. Figure 4 gives the ES-GC-MS profiles of the lavender e.o.analysed with the reference column. Table 5 reports enantiomer resolutions of optically active components, analysis time, % analysis time reduction and separation measure *S*, when lavender e.o.was analysed with the 10, 5 and 2 m NB columns, and compares them to those of the reference column. The 1 m NB column was not used, because the sample complexity was too high for its low efficiency. In this case too, the results were considered to be acceptable when all optically active components had a resolution above the limit of 1.5. These results also show that the 10m NB column can only be used at 2°C/min, because at higher rates terpinen-4-ol, linalyl acetate and borneol are not base-line separated. Figure 5a-b reports the ES-GC-MS profiles of the lavender e.o.analysed with the 5 m NB column at 5 and 10°C/min. The highest applicable temperature rate was 5°C/min, because at 10°C/min terpinen-4-ol resolution decreased to 1.2, as is clear from its ion profiles at m/z 71, 111, 154, in Figure5c-d. Under these conditions, the analysis time was reduced from about 43 min with the reference column to about 14 minutes.

The 2 m NB column gave an analysis time similar to that of the 5 m NB column (about 13 min at 5°C/min) and comparable enantiomer resolutions (see table 5). When one of the enantiomers of the markers co-eluted with other components, its resolution was calculated from their extracted ion MS profiles, selecting suitable diagnostic ions. Figure 6 reports the 2 m NB column ES-GC-MS profiles of the lavender e.o. together with those of extracted ion profiles of limonene (m/z 68, 107) linalool (m/z 80, 93) and borneol (m/z 95, 110) whose enantiomers in TIC mode coelute with those of other components. The use of shorter columns (2 and 5 m) results in lower values of the separation measure *S*, that however are still sufficient for a reliable separation of all lavender e.o. optically active markers. Table 6 reports the % area of each enantiomer calculated *vs.* the total area of the two enantiomers, and gives the relative standard deviation (RSD%) of three optically active components with different volatility, polarity and abundance (β-pinene, linalyl acetate and α-terpineol), analysed with the 5 m NB column at different temperature rates and with the 10, 5, and 2 m NB columns at the most effective temperature rate. These results show a high repeatability of enantiomer % areas under all conditions. % Areas of each enantiomer were chosen instead of EE or ER because it better shows repeatability under all conditions applied. The high RSD% of (*S*)-linalyl acetate is due to the noise interference with area determination because it is in trace amount.

The bergamot e.o. was analysed with the same NB columns and under the same conditions as those adopted for lavender e.o.. Five markers were selected, i.e. β-pinene, limonene, linalool, linalyl acetate and α-terpineol. This e.o. was chosen partly because it contains a highly predominant component (limonene) in its turn with a very high ER (EE), together with a set of minor markers important to define its quality. This situation is quite common in the field of real-world samples of natural origin. Narrow bore column capacity is lower than that of conventional I.D. columns, therefore the simultaneous analysis of all markers produces either (*R*)-limonene peak distortion or, with a suitably diluted sample, missing of minor components. The tailing of (*R*)-limonene produces a peak asymmetry of 5.4 with the reference column and of 3.9 and 6.8 for the 2 and 5 m NB columns, the late giving a reduction of *calculated* resolution below 1.5, although the two limonene enantiomers are more than baseline separated, as is clear from figure 7. A similar situation, though

with lower relative abundance, also occurred with linalool in lavender e.o.. In this case, the limonene resolution was calculated on the basis of the peak asymmetry value of (R)-enantiomer (see table 7). A series of analyses on the bergamot e.o. at different dilutions (from 1: 200 to 1:5000) also showed the linearity of (*R*)-limonene signal under the applied conditions (data not reported). Figure 7 gives the ES-GC-MS profiles of a sample of bergamot e.o. analysed with the reference column, and with the 2 and 5 m NB columns, together with the extract ion MS profiles of limonene (m/z 68) on the reference 2 m NB and 5m NB columns, required because of the coelution of ocimene and 2a limonene enantiomer on all columns, and of α-terpinene and 2b enantiomer on the 2 m NB column. Table 7 reports the enantiomer resolutions of five bergamot e.o. optically active markers, their analysis time, % analysis time reduction and *S* after analysis with the 10, 5, and 2m NB columns, analysed under different analytical conditions. In this case the limiting marker is linalyl acetate, whose enantiomers can only be separated at the base line at 2°C/min with the 10 m NB and up to 5°C/min with the 5 m NB column, while with the 2 m NB column they are separated in all the conditions. With the latter column the extract ion approach has only to be applied to limonene to obtain a correct EE or ER.

These results confirms those obtained with lavender e.o. analysis and show that 2 and 5 m NB columns can successfully be used to speed up bergamot e.o. analysis, affording a very marked reduction in analysis time: about 65% with the 5 m NB column at 5°C/min, and about 80% with the 2 m NB column at 10°C/min. The 2 and 5 m NB columns, at 5°C/min, gave separation measures (*S*) that were reasonably lower than those of the reference column (max 35%), thus enabling a temperature rate of 10°C/min to be applied to the 2 m NB column, with a further reduction in analysis time.

4. Conclusions

The results show that short conventional and narrow bore columns, combined with CD derivatives as chiral selectors, and MS as detector, can successfully be used for fast ES-GC analysis of real-world samples. These results have been obtained by adopting: 1) a resolution limit of 1.5 to enable a correct EE and ER determination; 2) mass spectrometry as detector to operate in both TIC and extract ion modes, to avoid problems due to co-elution with other components in the EE and ER determination. This assumption is correct because MS is not a chiral probe and the mass spectra of two enantiomers are indistinguishable; 3) temperature rates up to 10°C/min, in consideration that the enantioselective separations with CD as chiral selector are strongly conditioned by temperature, and 4) short conventional and narrow bore columns that not only contribute to shortening analysis time, but may also increase enantioselectivity and (at least partially) compensate for the loss of efficiency due to reduced length, which lowers elution temperature. The combination of these approaches enabled us to decrease analysis time by as much as 85% compared to that with conventional I.D. columns, while keeping a resolution suitable for a correct EE and ER determination, and to increase the number of samples analysable in a routine laboratory very markedly. These results also show that new CD derivatives with high enantioselectivity are still necessary not only to increase the number of optically active compounds separated or improve their resolution, but also to provide further speeding-up of ES-GC-(MS) routine analysis.

Further studies are under way to examine the influence of selectivity tuning with different CD derivatives as chiral selectors and diluting phases.

Acknowledgements

This research was carried out within the project entitled: ”Sviluppo di metodologie innovative per l'analisi di prodotti agroalimentari” (FIRB Cod.: RBIP06SXMR_002) of the *Ministero dell’Istruzione, dell’Università e della Ricerca* (MIUR) (Italy).

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Captions to figures

Figure 1: TIC ES-GC-MS profiles of α-HCH analysed with different 0.25 mm I.D. columns.

Figure 2: ES-GC-MS profile of linalool analysed with the four NB columns under investigation at 1 mL/min flow rate and 10°C/min temperature rate.

Figure 3: Resolution *versus* flow and temperature rates (A) and retention time *versus* flow and temperature rates (B) of γ-pentadecalactone on different columns

Figure 4: ES-GC-MS profile of the lavender essential oil analysed with the reference column (25 m x 0.25 mm I.D., 0.25 μm d_f). For analysis conditions see text. Peak identification: 1: α pinene, 2: camphene, 3: β-pinene, 4: β-phellandrene, 5: limonene, 6: 1-octen-3-ol, 7: camphor, 8: linalool, 9: borneol, 10: linalyl acetate, 11: 4-terpineol, 12: lavandulol, 13: α-terpineol, 14: lavandulyl acetate; a: *(R)* enantiomer, b: *(S)* enantiomer. 6: not separated; 7a and 12a: not detected;

Figure 5: ES-GC-MS profiles of the lavender e.o. analysed with the 5 m NB column at 5 (a) and 10°C/min (b). Extract ion profiles of terpinen-4-ol (71,111,154 m/z) at 5 (c) and 10°C/min (d). For analysis conditions see text. For peak identification, see caption to Figure 4.

Figure 6: 2m NB columns ES-GC-MS profiles of the lavender e.o. and extracted ion profiles of limonene, linalool and borneol. For analysis conditions see text. For peak identification, see caption to Figure4.

Figure 7: ES-GC-MS profiles of bergamot e.o. (top) and extract ion profiles of limonene (bottom) obtained with different columns and under different analysis conditions. Peak identification: 1: β pinene, 2: limonene, 3: linalool, 4: linalyl acetate, 5: α terpineol; a: *(R)* enantiomer, b: *(S)* enantiomer. %areas on total limonene area: (*S*)-limonene 2%, (*R*)-limonene 98% .

Table 1: Columns and analysis conditions adopted for the present study

Conventional (internal diameter 0.25 mm)				**Narrow bore (internal diameter 0.10 mm)**			
Columns		*Analysis conditions*		*Columns*		*Analysis conditions*	
Lenght (m)	Film thickness (μm)	Flow rate (mL/min)	Temperature rate (°C/min)	Lenght (m)	Film thickness (μm)	Flow rate (mL/min)	Temperature rate (°C/min)
25	0.25	1	2	10	0.10	0.4	2
25	0.15			5	0.10	0.7	3.5
5	0.15			2	0.10	1	5
				1	0.10		10

Table 3: Separation measure *S* calculated with the chiral test mixture under all conditions applied and for all columns investigated.

	S			
	2°C/min	3.5°C/min	5°C/min	10°C/min
Conv 25 m 0.25 µm	942			
Conv 25 m 0.15 µm	972			
Conv 5 m	491			
NB 10 m	477	442	395	347
NB 5 m	826	775	754	686
NB 2 m	727	705	697	562
NB 1 m	216	277	278	266

Table 4: % Areas of each enantiomer on the total areas of the *(R)*- and *(S)*-linalool enantiomers and related SD and RSD%, analysed with the 10, 5, 2, and 1 m NB columns under all conditions applied, and with the reference column.

Column	**% Area *(R)*/*(S)*-linalool**	**Mean**	**SD**	**RSD%**
Reference	*(R)*	**49.0**		
	(S)	**51.0**		
10 m (all anal cond.)	*(R)*	**48.9**	0.4	0.8
	(S)	**51.1**	0.4	0.7
5 m (all anal cond.)	*(R)*	**49.0**	0.4	0.8
	(S)	**51.0**	0.4	0.8
2 m (all anal cond.)	*(R)*	**49.5**	0.5	1.1
	(S)	**50.5**	0.5	1.1
1 m (all anal cond.)	*(R)*	**48.8**	0.4	0.7
	(S)	**51.2**	0.4	0.7
All columns and conditions	*(R)*	**49.1**	0.3	0.6
	(S)	**50.9**	0.3	0.6

Table 6: % Area of each enantiomer calculated *vs.* the total area of the two enantiomers of β-pinene, linalyl acetate and α-terpineol in lavender e.o., and related RSD%, analysed with the 5 m NB column at different temperature rates and with the 10, 5, and 2 m NB columns at the most effective temperature rate.

	%Area				Mean	RSD%
Column	**5 m**					
Temperature rate (°C/min)	**2**	**3.5**	**5**	**10**		
(+)-β-Pinene	56.3	54.7	54.3	54.5	**55.0**	**1.7**
(-)-β-Pinene	43.7	45.3	45.7	45.5	**45.0**	**2.1**
(R)-Linalyl acetate	99.5	99.7	99.6	99.6	**99.6**	**0.1**
(S)-Linalyl acetate	0.5	0.4	0.4	0.4	**0.4**	**12.7**
(S)-α-Terpineol	28.4	29.0	28.8	29.6	**28.9**	**1.8**
(R)-α-Terpineol	71.7	71.0	71.2	70.4	**71.1**	**0.8**
Column	**Ref.**	**10 m**	**5 m**	**2 m**	**Mean**	**RSD%**
Temperature rate (°C/min)	**2**	**2**	**5**	**5**		
(+)-β-Pinene	55.6	55.0	54.3	55.2	**55.1**	**1.0**
(-)-β-Pinene	44.4	45.0	45.7	44.8	**45.0**	**1.2**
(R)-Linalyl acetate	99.4	99.6	99.6	99.4	**99.5**	**0.1**
(S)-Linalyl acetate	0.6	0.4	0.4	0.6	**0.5**	**18.0**
(S)-α-Terpineol	27.5	28.7	28.8	28.2	**28.3**	**2.0**
(R)-α-Terpineol	72.5	71.4	71.2	71.8	**71.7**	**0.8**

Table 2: Retention time (R_t) of the second enatiomer, elution temperature of both enantiomers (T_{el}1-2), resolution (R) and % analysis time reduction (ATR) calculated on the second eluting enantiomer of the chiral standard compounds investigated.

	25 m x 0.2 5μm d_f			*25 m x 0.15 μm d_f*			*5 m x 0.15 μm d_f*			*ATR*	*ATR*	*ATR*
Compound	**t_R (min) II**	**T_{el} (°C) I-II**	**R**	**t_R (min) II**	**T_{el} (°C) I-II**	**R**	**t_R (min) II**	**T_{el} (°C) I-II**	**R**	***25 m 0.15μm vs 25 m 0.25 μm***	***5 m 0.15 μm vs 25 m 0.15 μm***	***5 m 0.15 μm vs 25 m 0.25 μm***
Limonene	18.67	85-87	8.2	11.72	72-73	8.4	3.66	57-58	4.1	37.2%	68.7%	80.4%
Linalool	25.15	99-100	7.9	18.77	86-87	7.8	7.94	65-66	4.2	25.4%	57.8%	68.4%
Linalyl acetate	27.80	105-106	3.0	20.60	90-91	3.7	8.76	67-68	2.5	25.9%	57.5%	68.5%
Linalyl propionate	31.78	113-114	1.0	24.15	98-98	1.2	11.16	72-72	1.0	24.9%	53.8%	64.9%
γ-Hexalactone	28.30	103-106	11.7	21.99	90-93	13.6	8.97	66-69	5.2	22.3%	59.2%	68.3%
γ-Heptalactone	32.50	112-115	11.9	26.04	99-102	13.9	13.08	73-76	5.2	20.6%	49.8%	62.7%
γ-Octalactone	36.73	121-123	10.0	30.05	107-110	11.6	16.69	80-83	4.8	18.2%	44.4%	56.7%
γ-Decalactone	46.06	141-142	5.7	38.96	126-128	7.2	24.88	98-100	3.8	15.4%	36.1%	46.0%
γ-Undecalactone	50.85	151-152	4.9	43.52	136-137	6.1	29.12	106-108	3.4	14.4%	34.2%	42.7%
γ-Dodecalactone	55.60	160-161	4.0	48.05	145-146	4.8	33.34	115-117	3.0	13.6%	33.1%	40.0%
γ-Tetradecalactone	65.41	179-180	2.4	56.82	163-164	3.2	41.34	131-133	2.7	13.1%	27.2%	36.8%
γ-Pentadecalactone	69.45	188-189	1.9	60.65	171-172	2.5	45.01	139-140	1.9	12.7%	25.7%	35.2%
δ-Octalactone	36.98	123-124	3.7	30.22	109-110	3.4	16.84	83-84	2.2	18.3%	44.3%	54.4%
α-HCH	29.00	156-158	5.9	21.09	140-142	8.8	7.60	114-115	5.3	27.3%	64.0%	73.8%
Trichlorfon	26.27	149-152	14.4	20.15	138-140	12.2	8.56	116-117	5.8	23.3%	57.5%	67.4%
trans-Chlordane	46.19	192-192	0.7	36.56	172-173	1.0	18.70	137-137	1.2	20.8%	48.9%	69.5%
Heptachlor	36.48	173-173	0.6	26.83	153-154	1.0	10.96	122-122	0.9	26.5%	59.2%	70.0%

Table 5: Enantiomer resolutions of optically active components, analysis time, % analysis time reduction and separation measure *S* obtained for a lavender e.o. analysed with the 10, 5 and 2 m NB columns in comparison to those of the reference column (Ref.).

		Ref.	10 m NB				5 m NB				2 m NB			
Temperature rate (°C/min)		**2**	**2**	**3.5**	**5**	**10**	**2**	**3.5**	**5**	**10**	**2**	**3.5**	**5**	**10**
Analysis time (min)		40.67	29.35	19.60	14.77	8.67	26.81	17.77	13.60	8.01	28.83	16.57	12.73	7.56
% Analysis time reduction			27.8	51.8	63.7	78.7	34.1	56.3	66.6	80.3	29.1	59.2	68.7	81.4
S		1077	407	374	333	286	840	808	744	652	759	701	688	582
	I*^{T}	**Resolution**												
α-Pinene (1)	*(R)*921/*(S)*923	1.2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Camphene (2)	(-)917/(+)923	6.8	2.2	1.9	1.8	1.7	6.7	5.6	5.6	4.9	3.9	3.5	3.4	3.5
β-Pinene (3)	(+)944/(-)955	5.0	1.8	1.5	1.4	1.2	5.3	4.8	4.4	3.5	3.3	3.3	2.9	2.6
β-Phellandrene (4)	(-)1049/(+)1060	6.1	2.1	1.8	1.7	1.5	6.2	5.3	5.1	4.5	3.1	2.8	2.6	2.4
Limonene (5)	*(S)*1056/*(R)*1072	9.1	2.8	2.5	1.9	1.9	9.0	8.2	8.0	5.0	5.4	4.2	3.8	2.9
1-Octen-3-ol (6)	1126	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Camphor (7)	*(S)*1133/*(R)*1141	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E
Linalool (8)	*(R)*1174/*(S)*1189	6.1	2.6	2.4	2.3	1.9	3.1	3.3	3.2	2.5	3.1	3.4	3.0	3.0
Borneol (9)	*(S)*1192/*(R)*1200	3.0	1.6	1.2	1.2	1.0	2.9	2.5	2.0	1.9	2.1	2.0	1.7	1.6
Linalyl acetate (10)	*(R)*1231/*(S)*1237	3.2	2.6	2.5	1.6	NS	3.8	3.0	2.8	2.4	4.1	3.8	3.1	1.9
Terpinen-4-ol (11)	*(S)*1248/*(R)*1253	2.2	1.6	1.0	NS	NS	2.4	2.1	2.0	1.2	2.6	2.3	2.0	1.3
Lavandulol (12)	*(S)*1250/*(R)*1273	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E
α-Terpineol (13)	*(S)*1296/*(R)*1309	6.0	3.2	2.8	2.5	1.9	6.9	5.6	5.5	4.2	6.5	5.6	4.7	4.0
Lavandulyl acetate (14)	*(R)*1259/*(S)*1263	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E	1E

NS: not baseline separated

1E: only *(R)* enantiomer found

*obtained on the reference column

Table 7: Enantiomer resolutions of five bergamot e.o. optically active components, analysis time, % analysis time reduction and separation measure *S* analysed with the 10, 5 and 2 m NB columns in comparison to those of the reference column (Ref.).

		Ref.	10 m NB				5 m NB				2 m NB			
Temperature rate (°C/min)		**2**	**2**	**3.5**	**5**	**10**	**2**	**3.5**	**5**	**10**	**2**	**3.5**	**5**	**10**
Analysis time (min)		41.81	30.62	20.04	15.24	8.90	28.11	18.47	14.07	8.23	26.11	17.28	13.20	7.76
% Analysis time reduction			26.8	52.1	63.5	78.7	32.8	55.8	66.3	80.3	37.5	58.7	68.4	81.4
S		1148	431	408	388	322	973	892	791	760	876	805	750	669
	I*^{T}*	**Resolution**												
β-Pinene (1)	(+)944/(-)955	4.0	1.2	1.1	1.1	1.0	3.1	2.9	2.8	2.7	2.3	2.3	2.2	2.0
Limonene (2)	*(S)*1056/*(R)*1072	4.8	1.2*	1.0*	1.0*	0.9*	3.8*	3.1*	2.8*	2.8*	2.2*	2.4*	2.2*	2.2*
Linalool (3)	*(R)*1174/*(S)*1189	6.7	3.0	2.8	2.6	1.7	5.2	5.1	4.6	4.3	4.8	4.2	4.2	3.6
Linalyl acetate (4)	*(R)*1231/*(S)*1237	3.0	1.5	0.9	NS	NS	2.6	2.4	1.9	0.9	2.8	2.7	2.5	1.9
α-Terpineol (5)	*(S)*1296/*(R)*1309	5.0	3.8	2.8	2.6	1.6	7.3	5.2	5.1	4.3	7.8	5.8	5.2	3.1

NS: not baseline separated

* calculated through peak asymmetry

**obtained on the reference column

Figure 1

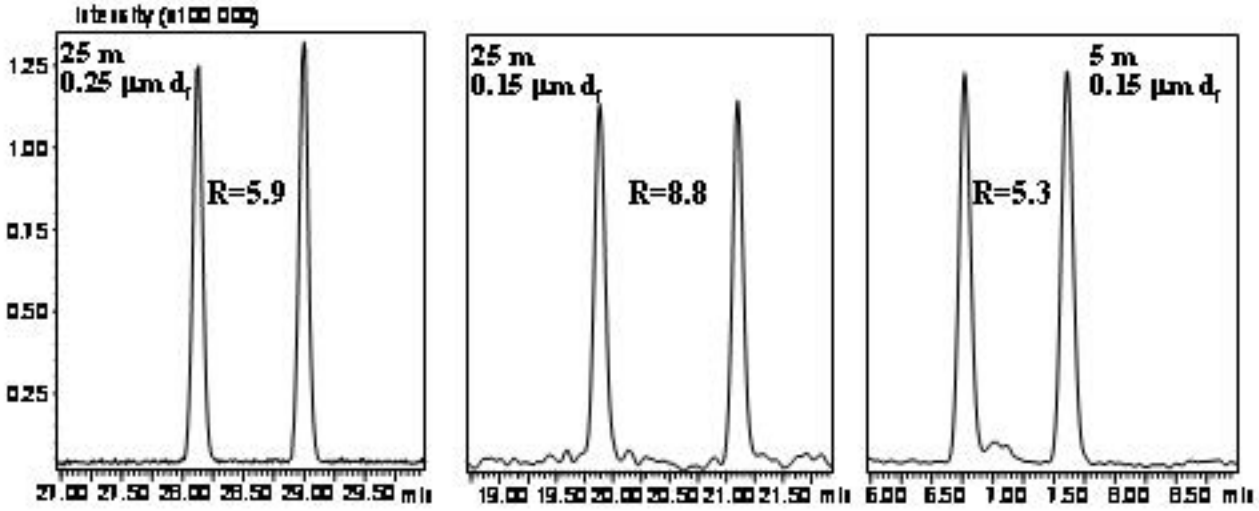


Figure 2

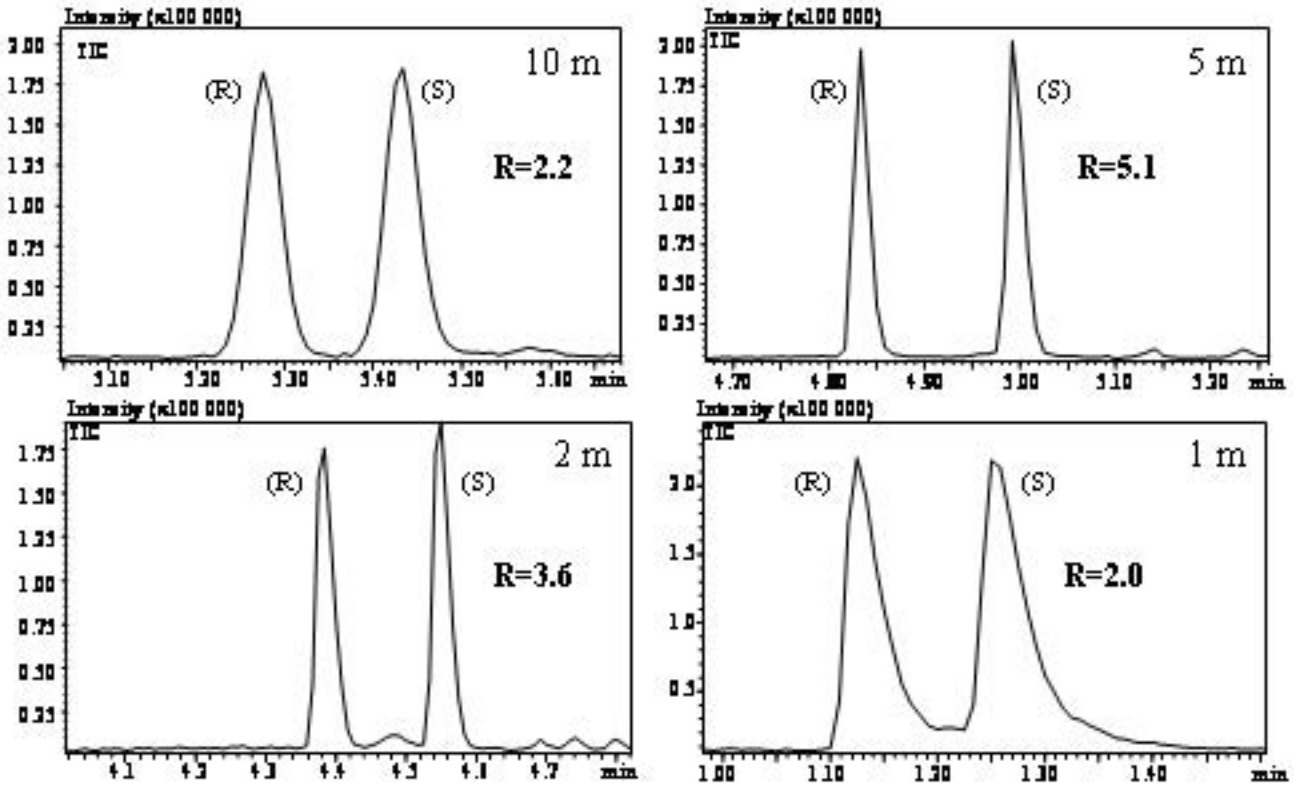
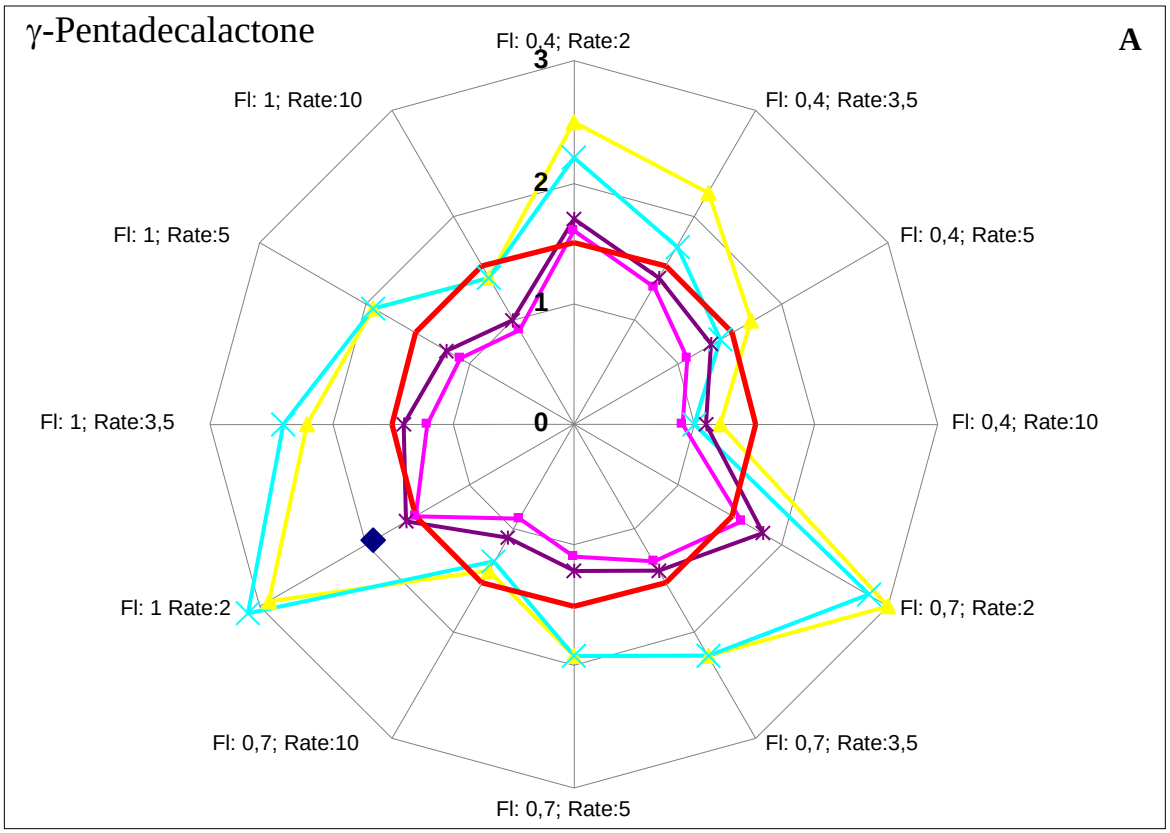


Figure 3


γ-Pentadecalactone
A
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Fl: 0,4; Rate:5
Fl: 0,4; Rate:10
Fl: 0,7; Rate:2
Fl: 0,7; Rate:3,5
Fl: 0,7; Rate:5
Fl: 0,7; Rate:10
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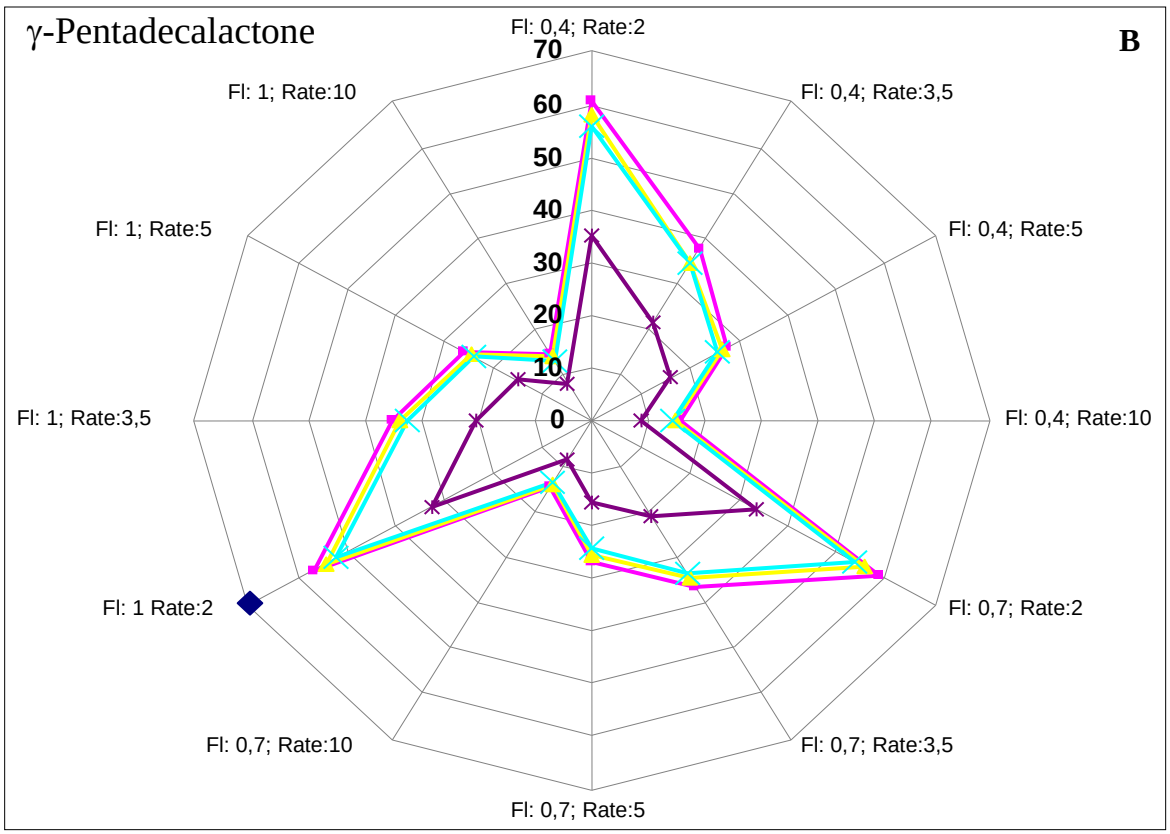
Fl: 1; Rate:5
Fl: 1; Rate:10
3
2
1
0


γ-Pentadecalactone
B
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Fl: 0,4; Rate:3,5
Fl: 0,4; Rate:5
Fl: 0,4; Rate:10
Fl: 0,7; Rate:2
Fl: 0,7; Rate:3,5
Fl: 0,7; Rate:5
Fl: 0,7; Rate:10
Fl: 1 Rate:2
Fl: 1; Rate:3,5
Fl: 1; Rate:5
Fl: 1; Rate:10
70
60
50
40
30
20
10
0
25mconv0.25
10m
5m
2m
1m
R=1.5

Figure 4

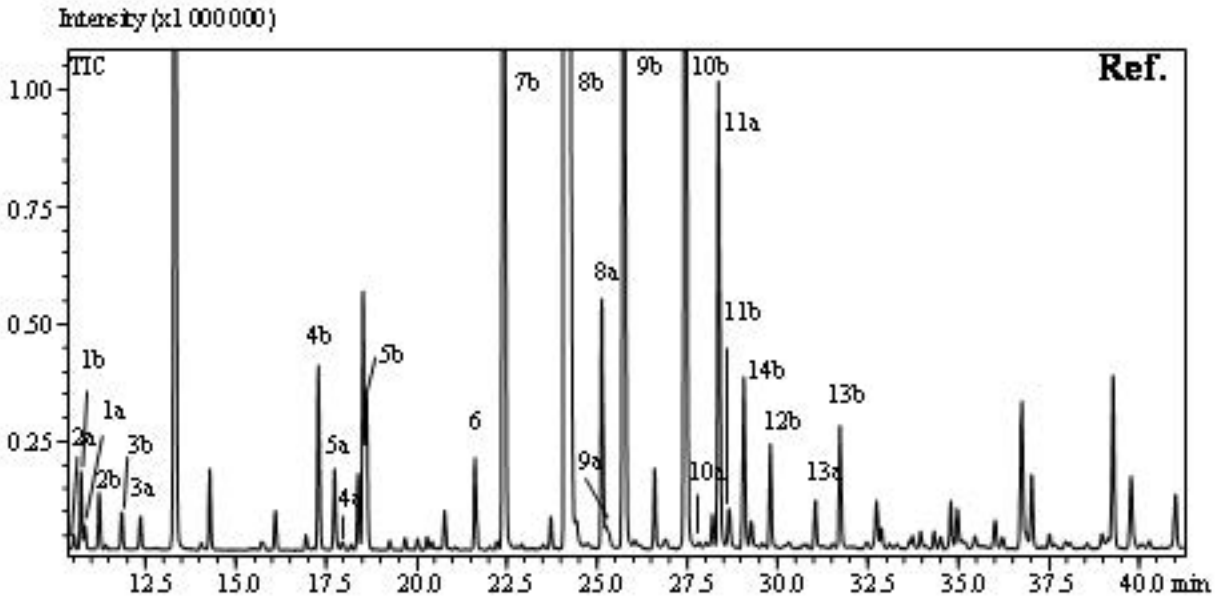

Intensity (x1 000 000)
TIC
Ref.
1.00
0.75
0.50
0.25
1b
1a
2a
2b
3b
3a
4b
4a
5a
5b
6
7b
8b
8a
9a
9b
10a
10b
11a
11b
12b
13a
13b
14b
12.5
15.0
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5
40.0 min

Figure 5

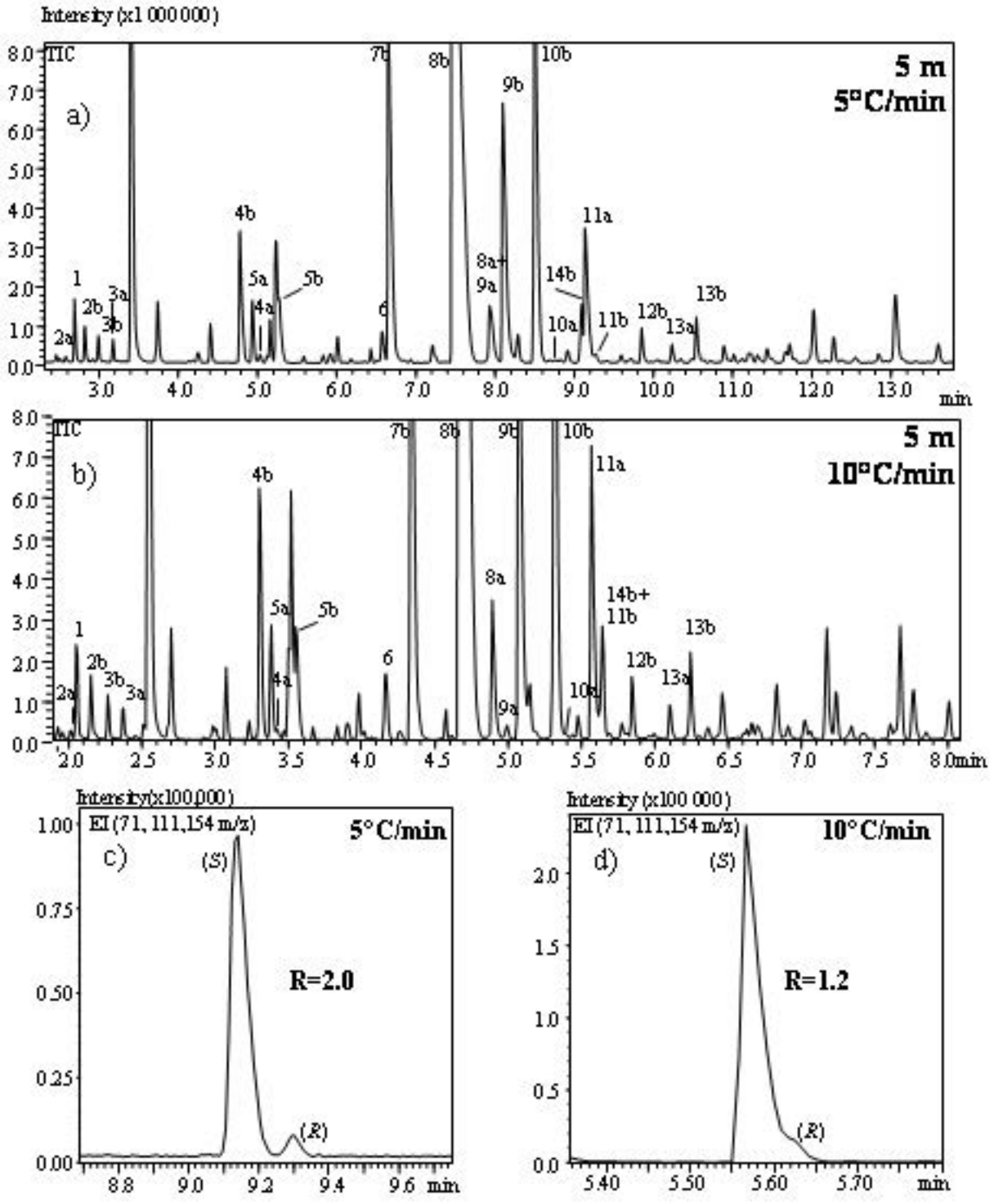

Intensity (x1 000 000)
TIC
a)
5 m
5°C/min
1
2a
2b
3a
3b
4b
5a
4a
5b
6
7b
8b
8a+
9a
9b
10a
10b
11a
11b
14b
12b
13a
13b
min
TIC
b)
5 m
10°C/min
min
Intensity (x100 000)
EI (71, 111, 154 m/z)
c)
5°C/min
(S)
(R)
R=2.0
min
Intensity (x100 000)
EI (71, 111, 154 m/z)
d)
10°C/min
(S)
(R)
R=1.2
min

Figure 6

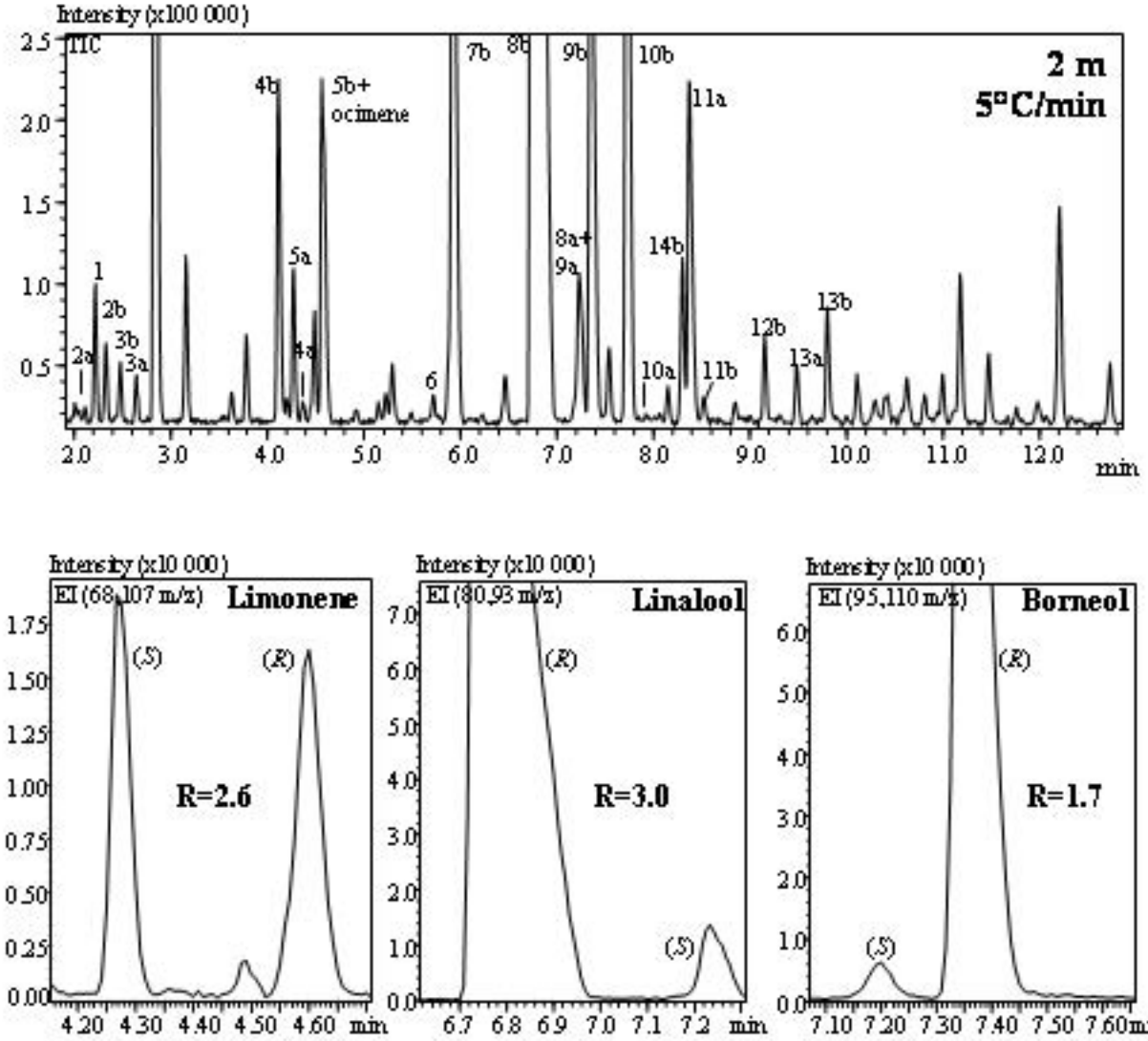
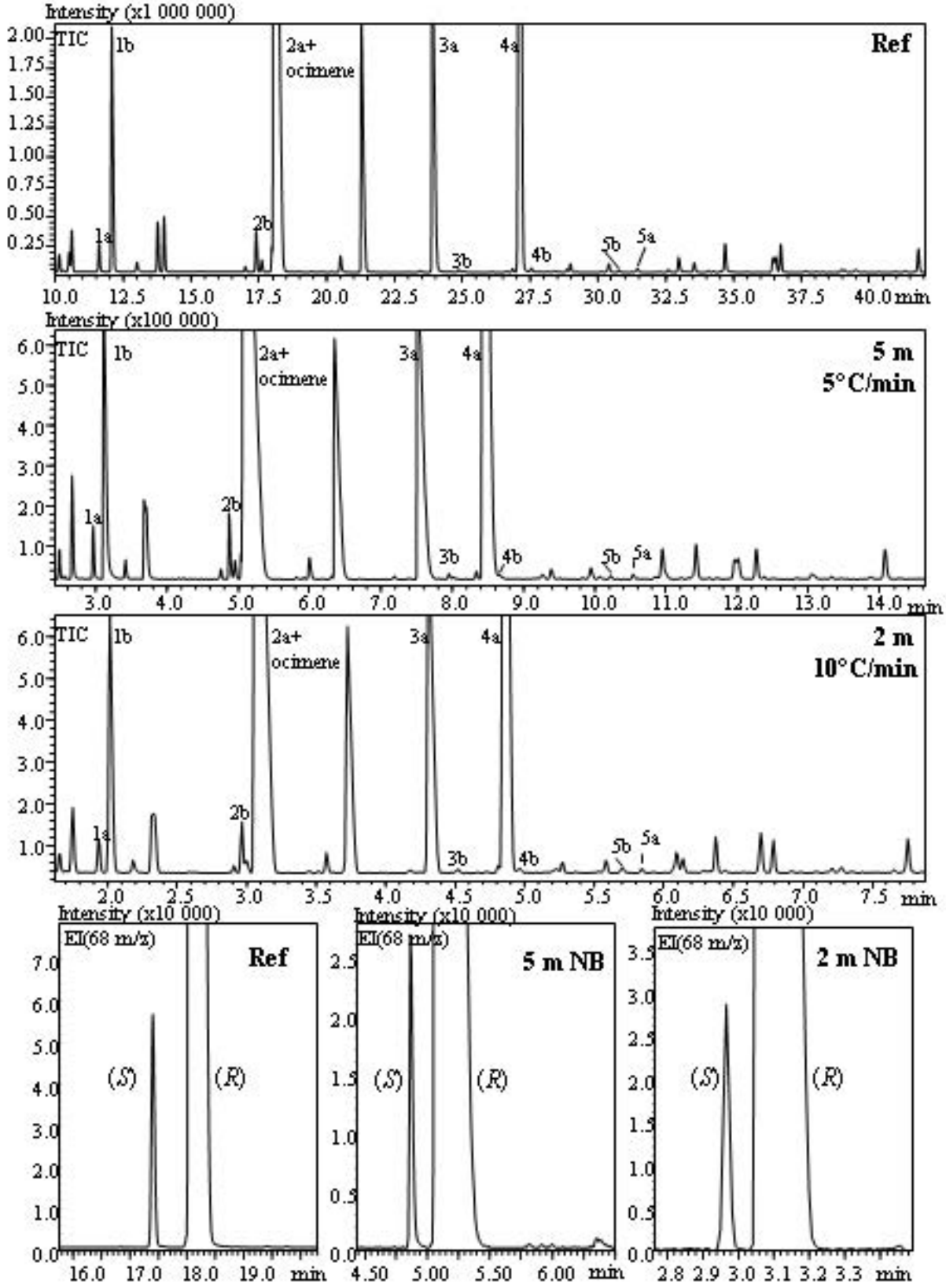

Intensity (x100 000)
TIC
2 m
5°C/min
1
2a
2b
3a
3b
4a
4b
5a
5b+
ocimene
6
7b
8b
8a+
9a
9b
10a
10b
11a
11b
12b
13a
13b
14b
min
Intensity (x10 000)
EI (68,107 m/z)
Limonene
(S)
(R)
R=2.6
Intensity (x10 000)
EI (80,93 m/z)
Linalool
(R)
(S)
R=3.0
Intensity (x10 000)
EI (95,110 m/z)
Borneol
(R)
(S)
R=1.7

Figure 7


Intensity (x1 000 000)
TIC
1b
2a+
ocimene
3a
4a
Ref
1a
2b
3b
4b
5b
5a
Intensity (x100 000)
5 m
5° C/min
2 m
10° C/min
Intensity (x10 000)
EI(68 m/z)
(S)
(R)
5 m NB
2 m NB
min