Conventional and enantioselective GC with microfabricated planar columns for analysis of real-world samples of plant volatile fraction

CONVENTIONAL AND ENANTIOSELECTIVE GC WITH MICROFABRICATED PLANAR COLUMNS FOR ANALYSIS OF REAL-WORLD SAMPLES OF PLANT VOLATILE FRACTION

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Abstract:

Within a project exploring the application of lab-on-chip GC to in-field analysis of the plant volatile fraction, this study evaluated the performance of a set of planar columns (also known as microchannels, MEMS columns, or microfabricated columns) of different dimensions installed in a conventional GC unit. Circular double-spiral-shaped-channel planar columns with different square/rectangular sections up to 2 m long were applied to the analysis of both essential oils and headspace samples of a group of medicinal and aromatic plants (chamomile, peppermint, sage, rosemary, lavender and bergamot) and of standard mixtures of related compounds; the results were compared to those obtained with reference narrow-bore columns (l: 5m, d_c: 0.1 mm, d_f: 0.1 µm). The above essential oils and headspaces were first analyzed quali- and quantitatively with planar columns statically coated with conventional stationary phases (5%-phenyl-polymethylsiloxane and auto-bondable nitroterephthalic-acid-modified polyethylene glycol), and then submitted to chiral recognition of their diagnostic markers, by enantioselective GC with a planar columns coated with a cyclodextrin derivative (30% $6^{I\text{-}VII}$-O-TBDMS-$3^{I\text{-}VII}$-O-ethyl-$2^{I\text{-}VII}$-O-ethyl-β-cyclodextrin in PS-086). Column characteristics and analysis conditions were first optimized to obtain suitable retention and efficiency for the samples investigated. The planar columns tested showed performances close to the reference conventional narrow-bore columns, with theoretical plate numbers per meter (N/m) ranging from 6100 to 7200 for those coated with the conventional stationary phases, and above 5600 for those with the chiral selector.

Keywords: micro-GC; planar columns; plant volatile fraction; essential oils; headspace sampling.

INTRODUCTION

Gas chromatography systems can be miniaturized either by scaling down each component (injection systems, columns, detectors) or by developing microfluidic devices with microelectromechanical systems (MEMS) technology, resulting in planar format [1, 2].

The first on-chip GC manufactured by silicon micromachining dates the late 1970s, and was developed by Terry et al. [3, 4]; it consisted of two wafers, the first containing an injection valve and a 1.5 m planar column, and the second a thermal conductivity detector. The state-of-the-art of on-a-chip gas chromatography, and its evolution over the last two decades, has very recently been extensively reviewed by Haghighi et al. [1 and references cited therein]. These authors offer a comprehensive and generalized overview of the entire field, in particular concerning materials and fabrication, chip-based injectors and pre-concentrators, planar columns and their geometry, and detectors. Terry et al. also first introduced planar columns (also known as microchannels, MEMS columns, or microfabricated columns) obtained with MEMS technology [3, 4]. Although this technology has evolved dramatically, a planar column [5] is in general prepared through a gas phase reactive ion etching process, that produces rectangular open channels on the silicon wafer substrate sealed with either a silicon or a Pyrex glass plate bonded to the silicon surface. In addition, planar column rapid heating through thin film resistance heating patterned in etched silicon channels has also been developed, to replace conventional convection ovens, thus saving energy and providing sufficiently rapid heating to increase the speed of separation with short columns [6-9]. Azzouz et al. [5] recently reviewed in depth the approaches to planar column technologies, the stationary phases adopted, and the fields in which they have been applied, in particular focusing on stationary phase incorporation in the packed or open tubular planar GC columns.

Compact instrumentation, offering technical characteristics and performance comparable to those of conventional setups, has obvious benefits in terms of saving energy, materials, and laboratory space. One of the areas where on-chip GC can play a fundamental role is in portable gas chromatographs for “in-field” analyses. In-field GC affords in-situ analysis immediately after (or on-line to) in-field sampling, thus avoiding sample alteration and markedly reducing analysis time. Successful applications have been reported in environmental control, the mineral oil industry, and in-field monitoring and control of toxic and explosive compounds, among others [1, 5].

The in-field and laboratory application to the volatile fraction emitted from plants is a field in which on-chip GC can offer interesting perspectives, and that has not yet investigated. On-chip GC, in particular if provided with an on-line connected sample preparation micro-concentration system, can successfully be used to monitor the evolution of the volatile fraction composition, and/or specific markers, for instance i) in plant processes such as maturation, ii) for in-lab and in-field studies of

ecological biochemistry and/or multitrophic phenomena, iii) in chemotaxonomy, and iv) in the on-line production of essential oils or extracts [10, 11]. The plant volatile fraction is in general a complex mixture, consisting of volatiles within a limited range of molecular weights, with fairly similar structures and physicochemical characteristics, relatively different polarities, and medium-to-high volatility; their analysis requires simple but very effective sampling techniques (e.g. headspace sampling) and highly efficient and selective analysis systems if meaningful separation is to be achieved. Similar considerations, although with different aims, can be made for samples in the flavor and fragrance field.

In view of a possible application of on-chip GC in these fields, this study aims to evaluate the performance of a set of planar columns of different dimensions, installed in a conventional GC, coated with different stationary phases, applied to the separation of sample components in the above fields, and compare their performances to those of conventional GC columns.

EXPERIMENTAL

Columns

Five planar columns of different geometry, coated with different stationary phases (SP), were tested. The stationary phases were 5%-phenyl-polymethylsiloxane (Sil-5%-PH) and auto-bondable nitroterephthalic-acid-modified polyethylene glycol (FFAP-EXT), and 30% $6^{I\text{-}VII}$-O-TBDMS-$3^{I\text{-}VII}$-O-ethyl-$2^{I\text{-}VII}$-O-ethyl-β-cyclodextrin in PS-086 (Et-β-CD).

The column characteristics are summarized in Table 1. The performances of the planar columns were compared to those of three conventional narrow-bore (NB) columns (l: 5m, d_c: 0.10mm, d_f: 0.10 μm) coated with the same stationary phases (i.e., Sil-5%-PH, FFAP-EXT, and Et-β-CD).

Preparation of the planar columns: The micromachined circular double-spiral-shaped channel planar columns (Figure 1) were fabricated using silicon direct bonding of two wafers (1.5 x 1.5 cm^2): on one wafer, rectangular (or square) channels were etched using a Deep Reactive Ion Etching (D-RIE) with a silicon oxide mask layer. Total column length and dimensions of the etched channels are in Table 1. On the second wafer, inlet and outlet holes were bored using D-RIE. Lastly, after wafer bonding and singulation, standard fluidic interconnections were made on the chip, by inserting deactivated fused silica capillaries (l: ca. 25cm long, d_c: 0.1 mm) into the inlet/outlet holes and sealing them with a polyimide resin. As a term of comparison, a nominal channel inner diameter (nd_c) for each planar column to be used was calculated through the following expression: $nd_c = 2(S/\pi)^{1/2}$ where S is the area of the channel section.

Static coating of the planar columns:

The planar columns were statically coated using the following procedures.

Apolar and chiral columns: the planar silicon column was first rinsed with an HCl solution and then dried under a N_2 flow. A solution of hexamethyldislazane (HMDS) in pentane was then passed through the column, then sealed under N_2 and heated in a GC oven to accomplish the deactivation step. The column was rinsed with methanol and diethyl ether in series and again dried under a N_2 flow.

The column was then filled with a solution of the stationary phase at a concentration suitable to obtain a nominal film thickness equivalent to that of a circular column, and submitted to the static coating process (Table 1)

Polar column: the same acid treatment as for the apolar and chiral columns was applied to the planar column. The column was then immediately filled with a solution of autobonding FFAP-EXT stationary phase at a concentration suitable to obtain the required nominal film thickness (Table 1).

All columns were from MEGA (Legnano (MI), Italy).

Samples

Five standard mixtures were analyzed: i) a Grob test [12], ii) a chiral test developed in the authors' DSTF laboratory, consisting of ten compounds with different structures and polarities (limonene, 2-octanol, camphor, isobornyl acetate,linalyl acetate, 2-methyl-(3*Z*)-hexenyl butyrate, menthol, hydroxycitronellal, γ-decalactone and δ-decalactone racemates [13]), iii) a "linalools" standard mixture, consisting of racemic linalool, linalyl acetate and linalyl propionate, iv) a "menthols" standard mixture, consisting of racemic menthone, isomenthone, menthyl acetate, menthol, neomenthol, isomenthol and neoisomenthol, and v) a standard mixture of racemic C_6 to C_{12} γ-lactones. The essential oils of lavender (*Lavandula angustifolia* Mill.), bergamot (*Citrus limon* (L.) Osbeck), peppermint (*Mentha* x *piperita* L.), rosemary (*Rosmarinus officinalis* L.), chamomile (*Matricaria chamomilla* L.), and sage (*Salvia officinalis* L.) were also analyzed.

All standard compounds were from the collection of standards in the authors' laboratory or, if unavailable there, were obtained from Sigma–Aldrich (Milan, Italy); they were dissolved in cyclohexane at a concentration of 0.1 mg/ml each. The essential oils, obtained by hydrodistillation following the European Pharmacopoeia (8th edition) [14], were solubilized in cyclohexane at a concentration of 5 mg/ml before analysis. Solvents were all HPLC grade from Sigma–Aldrich (Milan, Italy).

Instrumental set-up

142 Analyses were carried out on a Shimadzu GC-FID 2010 system provided with Shimadzu GC Solution
143 2.53SU software (Shimadzu, Milan, Italy). Temperature for injector: 220°C, temperature for detector:
144 230°C; carrier gas: H_2, flow control mode: constant linear velocity, FID sampling rate: 40msec.
145
146 ***Analysis conditions***
147 Column performances were evaluated by analyzing the Grob test isothermally at 100°C at different
148 flow rates, ranging from 0.1 ml/min to 0.5 ml/min. The plate number (N) was calculated at each flow
149 rate on ethyl dodecanoate using the following equation $N=5.54*(t_R/fwhm)^2$ where t_R is the retention
150 time, and fwhm is the full width at half-maximum. The height equivalent to a theoretical plate *(H=N/L*
151 were *L* is the column length) at each flow rate was also calculated to build the Van Deemter curves
152 for each column and, as a consequence, to determine the flow rate corresponding to the minimum
153 relative plate heights (*h*), i.e. the efficiency optimized flow (EOF) [15].
154 The Et-β-CD MEMS columns were tested by analyzing the chiral test, the "linalools", the γ- lactones
155 standard mixtures, and the lavender and bergamot essential oils at the EOF of each column and at
156 different temperature rates (i.e. 2, 3, 5, 7.5, 10, 15°C/min)
157 The Sil-5%-PH and FFAP-EXT MEMS columns were tested by analyzing the "menthols" standard
158 mixture and the peppermint, rosemary and sage essential oils at the EOF of each column and at
159 different temperature rates (i.e. 2, 3, 5, 7.5, 10, 15°C/min).
160 In all cases, the maximum operative temperature was limited to 190°C, to avoid [problems with]
161 damaging the chip/tubing connection.
162 The headspace (HS) analysis of the volatile fraction of chamomile and rosemary was carried out by
163 headspace solid phase microextraction (HS-SPME), sampling 20 mg of the plant material in a 20 ml
164 sealed vial with a 2 cm-DVB/Carboxen/PDMS fiber for 30 minutes, at i) 50°C for the rosemary
165 sample and at ii) 80°C for the chamomile sample.
166 Individual peaks were identified by comparing their linear retention indices calculated versus a C9–
167 C25 hydrocarbon mixture to those of authentic samples, as well as by comparing their mass spectra
168 to a set of commercial and in-house libraries. Percentage composition was determined from GC-FID
169 data through the peak area normalization approach, adopting a response factor for each class or sub-
170 class of compounds (hydrocarbons, aldehydes, alcohols, esters, etc.) in the investigated sample,
171 calculated versus the internal standard, taking one component representative of each class [16].
172

173 ***Quantitation of 1,8-cineol and camphor in rosemary essential oil***

174 Stock standard mixtures of 1,8-cineol and camphor were prepared by adding an aliquot of pure
175 standard to an appropriate volume of cyclohexane at an initial concentration of 5 mg/ml. Suitable
176 dilutions (5-7) of each stock standard mixture in cyclohexane were then prepared in the concentration
177 range (5-0.1 mg/ml). The resulting solutions (stock and diluted) were stored at 0°C and renewed
178 weekly. Each calibration solution was analyzed in triplicate by GC-FID with columns 1 and Ref. 1,
179 under the conditions reported in the previous paragraph, and the 1,8-cineol and camphor calibration
180 curves were built. The rosemary essential oil solution diluted in 5 mg/ml in cyclohexane was analyzed
181 using isobutylmethylketone as internal standard.

182

183 ***3. RESULTS AND DISCUSSION***

184 This study aimed to evaluate whether planar columns can successfully be applied to the analysis of
185 samples of the volatile fraction of different aromatic plants, in the form of headspace and of essential
186 oil, and to compare separation results with those of conventional narrow bore (NB) columns coated
187 with the same stationary phases; this was in view of their possible application in micro GC systems
188 for in-field analysis. In consideration of the complexity of the samples investigated, mainly consisting
189 of isomeric components, or of components within a limited range of molecular weights and with
190 similar volatilities, separation was considered to have priority over analysis time.

191 The volatile fractions to be investigated were obtained through the two most widely used sample
192 preparation methods for plant volatile fractions, i.e. essential oil hydrodistillation and headspace
193 sampling; the latter approach was applied partly in view of the possible development of a
194 microconcentrator to be integrated into a micro-total analysis system (μTAS) based on-chip GC [1].
195 These two methods are based on different principles (hydrodistillation and vaporization) but they
196 both represent this fraction equally well, although they cannot be directly quali-quantitatively
197 compared. Essential oils of different compositions, dry plants, and standard mixtures of critical pairs
198 of analytes, were then used to test the applicability of planar-columns to this field.

199 A set of five planar columns of different lengths, diameters, and film thicknesses, statically coated
200 with the stationary phases (SP) commonly adopted to analyze the plant volatile fraction, were
201 investigated, namely, dimethyl polysiloxane 5 % phenyl (Sil-5%-PH) and acid-treated polyethylene
202 glycol (FFAP-EXT) as apolar and polar SPs for conventional GC. A 30% $6^{I\text{-}VII}$-*O*-TBDMS-$3^{I\text{-}VII}$-*O*-
203 ethyl-$2^{I\text{-}VII}$-*O*-ethyl-β-cyclodextrin in PS-086 (Et-β-CD) SP was used as chiral selector for
204 enantioselective GC (Es-GC) [17]. In addition, FFAP-EXT, Sil-5%-PH, and Et-β-CD NB columns
205 (l: 5 m, d_c: 100μm; d_f: 0.1 μm (Table 1) were adopted as reference. The characteristics and
206 dimensions of both the investigated MEMS planar columns and the reference 5m NB columns are

summarized in Table 1. Particular attention was paid to film thickness, because thicker SP film increases analyte retention, thus overcoming one of the limits of short columns with very volatile compounds. Table 1 also gives the nominal film thickness and the concentration of the SP coating solutions, since it is known that the non-circular shape of the microchannels leads to a distribution of SP film layer across the section of the column that is not entirely homogenous, even with the static coating process [5].

3.1 Column performances

The performance of all planar and reference columns investigated was first tested through the Grob test [12] to evaluate the effectiveness of the static coating process in terms of inertness, efficiency, and retention. Planar column preparation had therefore to be adapted to the nature of the silica surface of the microchannels resulted from the etching process (see experimental section). The static coating process for rectangular planar columns (150x240 µm, 25-300 cm long) coated with different PDMS film thicknesses (0.1-0.2 μm) has been investigated in depth by Reidy et al. [18]. The resulting in situ cross-linked planar columns provided over 4000 theoretical plates/m, using air as carrier gas, determined with n-octane in isothermal conditions, i.e. ca.12,500 theoretical plates for a 3.0 m column, and a peak capacity of over 100 measured with a n-C5 to n-C12 alkane mixture in temperature-programmed mode (10 °C/min).

The Grob test clearly showed that the conditions adopted for column preparation provided inert and effective columns, with a theoretical plate number per meter (N/m) ranging between 6100 and 7200 for conventional SP, and above 5600 for chiral SP (Table 1) depending on column-length, film thickness and inner diameter. N/m was calculated isothermally (100°C) on ethyl dodecanoate, using hydrogen as carrier gas at the optimal column head-pressure. These values are comparable to, or only reasonably lower than, those of the reference narrow-bore columns; this loss of efficiency is probably due to the extra-column band broadening produced by the planar column connections to the injector and detector of the conventional GC units [18].

These results were confirmed by the Van Deemter plots. Figure 2 reports the Van Deemter plots of the five planar columns and of the corresponding reference NB columns, and Table 1 gives the parameters characterizing column performances (i.e. N, N/m, and *S*). The Van Deemter curves confirm that the FFAP-EXT and Sil-5%-PH MEMS planar columns were slightly less effective than those of the corresponding reference NB columns, but nevertheless comparable to them. Conversely, the Et-β-CD MEMS planar columns performed differently, i.e. column 5 presented a Van Deemter curve that almost overlapped that of the corresponding reference NB column, while the performance of column 4 was decidedly poorer.

3.2. Analysis of the volatile fraction of aromatic plants by conventional GC

The first example concerns the analysis of the chamomile (*Matricaria chamomilla* L.) volatile fraction, whose composition mainly consists of sesquitepenoids. The analysis was carried out on both its e.o., obtained by hydrodistillation, and on its headspace, sampled by SPME; it was analyzed on the thin-film FFAP-EXT planar column (column 2). Figure 3 reports the GC profiles (a) of the chamomile e.o., and (b) of the headspace from the same plant material sampled by SPME; profiles were obtained with both the thin-film FFAP-EXT planar column (column 2) and the reference NB column (Ref 2). The planar column provides a separation that perfectly overlaps that of the conventional NB column, enabling a following correct quantitation of all chamomile markers. The fairly high volatility of the components of the chamomile e.o., which are all sesquiterpenoids, means that a thin-film planar column can be used in spite of its relatively low retention, and a high temperature rate can be used, giving a short analysis time without affecting separation.

The second example in this section concerns the analysis of peppermint essential oil, and the separation of a critical mixture of menthol derivatives and isomers that characterizes it. The standard mixture of menthol derivatives and isomers was very well separated with the thin-film FFAP-EXT planar column at 2°C/min (data not reported). Analysis time could be shortened by increasing the temperature rate; however, faster analysis conditions could not be transferred to peppermint e.o. analysis, because peaks eluting with retention times shorter than menthone would coelute with the solvent peak, owing to the low retention power of the column. In any case, even with the temperature rate adopted, the most volatile e.o. components eluted together with the solvent peak. The coeluting compounds are chiefly monoterpene hydrocarbons (e.g. pinenes and limonene) and slightly polar oxygenated components (e.g. 1,8-cineole), which play an important role in defining the quality of peppermint e.o..

By using a FFAP-EXT planar column coated with film of double nominal thickness, a higher temperature rate (15°C/min) could be used with comparable separation, while analysis time could be reduced by 70% on the menthol standard mixture. Under these conditions, the thicker SP film produced an increased retention of the early-eluting components, avoiding coelution of limonene and 1,8-cineole with the solvent peak. However, even with this planar column under these conditions, α- and β-pinenes and related components coeluted with the solvent, meaning that either a longer column coated with the same SP, and/or a different SP, would be required to achieve meaningful peppermint e.o. analysis. Figure 4 reports the GC profiles of the menthol derivatives and isomers standard mixture, obtained with (A) FFAP-EXT planar column 3 at 15°C/min, and (B) Sil-5%-PH planar column 1.

Conversely, a satisfactory GC profile of peppermint e.o. was obtained with a different SP, i.e. a Sil-5%-PH planar column (50x50 µm; l: 2m; nominal d_f: 0.28 µm) (column 1) that gave good separation not only of the menthol derivatives and isomers, but also of the highly volatile e.o. fraction from the solvent, α- and β-pinenes and related components included. As expected, the analysis time increased to about 30 minutes, partly in consequence of the relatively high film thickness and low temperature rate (2°C/min). Figure 5 reports the GC profiles of peppermint e.o. obtained with (A) FFAP-EXT planar column 3, (B) Sil-5%-PH planar column 1, and (C) the reference Sil-5%-PH NB column (Ref. 1). In this case, stationary phase selectivity played the crucial role in obtaining the necessary retention and separation; the results with column 1 were perfectly comparable, if not better, than those obtained with the conventional NB column.

The next example concerns sage (*Salvia officinalis* L.) e.o., and evaluates the reliability of the qualitative chromatographic information (i.e. linear retention indices (I^T_S) [19]) that can be achieved with planar columns, and compares them to those obtained with the corresponding conventional NB column. I^T_Ss are a fundamental tool for component identification, in particular in in-field analysis, since the combination of micro-GC with micro-mass spectrometry is still at the early stages of development. I^T_S are especially important with the plant volatile fraction, which often consists of several isomers belonging to the same class of secondary metabolites (e.g. monoterpenoids) with similar chromatographic performances. Sage e.o contains a series of terpenoids belonging to different groups, ranging from monoterpene hydrocarbons (C10, e.g. α- and β-pinene) to diterpenic alcohols (C20, e.g. sclareol). Sage e.o. was analyzed using a Sil-5%-PH planar and a NB column, measuring the I^T_S *versus* a C9-C25 hydrocarbon standard mixture. The analysis conditions were optimized on the planar column, and translated to the reference NB column by the translation approach method [15]. Figure 6 reports the GC profiles of the sage e.o., obtained with (a) the Sil-5%-PH column 1, and (b) the corresponding NB column, while table 2 reports retention indices and percent abundance of its components, normalized to ethyl dodecanoate. These results show that the chromatographic performances are highly comparable, both in qualitative terms (I^T_S varying by a maximum of 7 units) and in terms of normalized peak areas (RSD on the area percent being below 10% in all cases).

The last example concerns the quantitative analysis of the diagnostic markers one of the chemotypes of rosemary e.o. (*Rosmarinus officinalis* L.). This species is characterized by several chemotypes, whose chemical composition strongly influences its organoleptic properties; the main components of the sample investigated were 1,8-cineol and camphor, together with some minor but important monoterpene hydrocarbons, in particular, α- and β-pinene. As for peppermint e.o., the Sil-5%-PH planar column was used (column 1: 50x50 µm; l: 2m; nominal d_f: 0.28 µm) and its results compared to those of the corresponding NB column (Ref. 1). Figure S1 gives the GC profiles of the rosemary

e.o. obtained (a) with the thick-film Sil-5%-PH planar column (column 1) and (b) with the corresponding reference NB column. With both columns, linearity was very good (for camphor: column 1 r^2: 0.9996; ref. 1: r^2:0.9998, and for 1,8-cineol: column 1 r^2: 0.9981; ref. 1: r^2:0.9984). The quantitative results were also very satisfactory, accounting for 355 mg/g 1,8-cineol with ref 1, and 370 mg/g with column 1 (RSD% = 2.93%), and 235 mg/g of camphor with ref. 1 and 229 mg/g with column 2 (RSD% = 1.83%).

3.3 Enantioselective GC with planar columns

A further fundamental step in studying the plant volatile fraction is chiral recognition, using enantioselective GC to separate the enantiomers of chiral compounds, and to determine their enantiomeric excess (ee) and/or ratio (er). This is useful, among others, (i) for defining the biosynthetic pathway of a given compounds characterizing a matrix; (ii) for determining the geographic origin of e.o.s or plant materials, (iii) for implementing quality control of plant material and detecting e.o. frauds or adulteration, and (iv) for correlating chemical composition to organoleptic properties, in particular for applications in the flavor and fragrance field [20, 21]. Highly effective chiral stationary phases (CSP), such as cyclodextrin derivatives, are necessary for the chiral recognition of markers in complex real-world samples, in particular when applied to planar columns. The separation of enantiomers by ES-GC, with CD derivatives as CSP, is based on fast kinetics and thermodynamically driven; the resulting low elution temperature are in favor of a successful enantiomer separation [22]. The short length of planar columns facilitates low elution temperatures, thus increasing enantioselectivity, and their small nominal inner diameter compensates (in full or in part) for the loss of efficiency (N) due to column shortening.

A first set of experiments was carried out with a thin-film Et-β-CD in PS086 MEMS, column 50x50 µm, nominal inner diameter 56 µm, l: 1.68 m, nominal d_f: 0.09 µm . As shown in Figure 2, the efficiency of this column was relatively low (about 2200 theoretical plate/m) most probably because of the non-uniform film due to the mixed cyclodextrin/PS-086 SP, when coated on small-section microchannels. In consequence, its enantioselectivity was also rather poor, as shown by the separation of the test mixture of linalool, linalyl acetate and linalyl propionate (Figure 2aSM). These three compounds are good diagnostic markers of the enantioselectivity of a chiral selector, because, in spite of their closely related structures, they interact differently with the CD/diluting phase chiral probe: linalool is well separated with almost all CD probes, linalyl acetate is well separated only with specific CD derivatives such as Et-β-CD, and linalyl propionate is poorly separated with all CD derivatives. The enantioselectivity of this column was only sufficient for a limited number of chiral compounds, i.e. those whose enantiomers present a big difference in host/guest interaction energy with the CD

343 chiral selector. This is the case of the test mixture of gamma lactones C6-C12, which were well
344 separated on this column (Figure 2bSM). The performance of this planar column was not sufficient
345 for applications to real-world medium-complex mixtures, such as a plant volatile fraction and/or e.o.s,
346 because it lacked efficiency, raising the risk of peak overlapping.
347 A new planar column (column 5: 80x80 µm - nominal d_c: 91 µm, l: 2m, nominal d_f: 0.18 µm) was
348 thus tested. The increased column length and film thickness provided better efficiency, as shown in
349 Figure 2, with more than 5600 theoretical plates per meter (N/m) and almost twice the separation
350 measure of the previous column (462 vs. 247).
351 This column presented good enantioselectivity and chromatographic performance. Figure 7 compares
352 the Es-GC profiles obtained with planar column 5, of (A) the chiral test, (B) the mixture of γ-lactones
353 C6-C12, and (C) the test mixture of linalool, linalyl acetate and linalyl propionate, with the same
354 samples analyzed with the Ref 3 column.
355 The chiral test showed a profile that was closely comparable to that of the reference 5 m NB column,
356 even at a reasonable temperature rate, that limits the analysis time to about 15 minutes. Positive results
357 were also obtained with the linalool, linalyl acetate and linalyl propionate standard mixture, in which
358 to obtain a partial separation (about 40%) of linalyl propionate, the rate had to be limited to 3°C/min.
359 The analysis time can, of course, be drastically reduced at the expenses of the separation of those
360 enantiomers that interact weakly with the CD chiral selector. Conversely, all components of the C6-
361 C12 γ-lactone homologous series are baseline separated even at 10°C/min, because of its strong host-
362 guest interaction with the CD derivative, enabling an analysis time of about 11 minutes to be achieved.
363 The enantiomeric ratios of linalool and linalyl acetate are parameters diagnostic of the authenticity of
364 two medium-complexity e.o.s, i.e. lavender and bergamot e.o.s [23, 24]. Two samples of these e.o.s
365 were therefore directly analysed with the above MEMS column, under the analysis conditions
366 reported above. Figure 8 shown the Es-GC-FID profiles of (A) a lavender e.o. at a rate 5°C/min and
367 (B) a bergamot e.o. at a rate of 5°C/min. The resulting Es-GC profiles are closely comparable to those
368 obtained with a 5 m NB column, and can therefore provide a correct and direct determination of both
369 ee or er of linalool and linalyl acetate in the two investigated e.o.. This means that the “one column
370 for one problem” approach described elsewhere can also be applied to planar columns [25].
371

4 CONCLUSIONS

The results reported here show that planar columns can successfully be used for the in-field analysis of the plant volatile fraction by a lab-on-chip GC; all aspects required to characterize a plant volatile fraction (i.e. peak identification and quantitation, and chiral recognition) may be covered. Column performances have been shown to be compatible with those required for a correct analysis of the plant volatile fraction, and comparable to those of conventional narrow-bore columns; the effectiveness of the static coating procedure has also been demonstrated. These results effectively contribute to defining optimal nominal inner diameter, film thickness, and column length, so as to optimize planar column performance in view of application in this field. For this purpose, a suitable injection system consisting of a planar microconcentrator to be connected on-line to the planar column is under study. In a final lab-on-chip GC configuration, the planar columns will not be inserted into a GC oven and interconnected to injectors and detectors using uncoated fused silica capillaries, but preferably packaged at chip level with planar MEMS injectors and detectors and heated on-chip using thin-film metal heating resistors. This would significantly contribute to increase planar column efficiency because of the drastic reduction of the void volumes.

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394

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458
459

460 **Tables**

461 *Table 1* – List of planar and reference NB columns used in this study, together with their
462 characteristics and performances.

463 Nom. d_c*:* nominal inner diameter; Nom d_f: nominal film thickness; Coating SP conc.: coating
464 stationary phase concentration; N: total number of theoretical plates; N/m: number of theoretical
465 plates per meter; *S*: separation measure calculated on peppermint e.o. for polar and apolar columns
466 and on chiral test for chiral columns; EOF: efficiency optimized flow; Linear vel.: linear velocity

467

Column #	Phase	Length (cm)	Width (µm)	Depth (µm)	Nom. d_c (µm)	Nom. d_f (µm)	Coating. SP conc. (mg/10mL)	N	N/m	S	EOF mL/min	Linear vel. cm/s	Pressure Kpa
1	Sil-5%-PH	200	50	50	56	0.28	200	13512	6756	463	0.4	114.7	203
Ref.1	Sil-5%-PH	5m	circ 100		100	0.10	40	37151	7430	641	0.4	59.9	90.8
2	FFAP-EXT	168	50	80	71	0.10	56	10263	6109	270	0.4	104.1	111.5
3	FFAP-EXT	168	50	80	71	0.22	120	12209	7267	419	0.4	104.1	112.5
Ref.2	FFAP-EXT	5m	circ 100		100	0.10	40	37516	7503	598	0.4	59.9	90.8
4	Et-β-CD	168	50	50	56	0.06	40	3678	2189	247	0.3	40.4	19.8
5	Et-β-CD	200	80	80	91	0.18	75	11290	5645	462	0.4	74.8	45.6
Ref.3	Et-β-CD	5m	circ 100		100	0.10	40	28952	5790	711	0.4	48.4	150.9

468

469 *Table 2* - Comparison of linear retention indices (I^T_S) of marker components of a *Salvia officinalis*
470 e.o analyzed by GC with a planar column (column 1) and a reference NB column (ref 1).

	Compound	I^T_S Col.1	I^T_S Ref.1	ΔI^T_S	Area% Col.1	Area% Ref.1	RSD Area%
1	α-pinene	919	920	1	2.38	2.37	0.30
2	camphene	928	931	3	2.41	2.46	0.94
3	β-pinene	954	959	5	2.63	2.70	1.25
4	β-mircene	978	984	6	0.85	0.82	1.45
5	*p* cymene	1004	1010	6	0.66	0.70	2.69
6	1,8-cineole	1008	1014	6	7.22	7.57	2.34
7	limonene	1011	1016	5	1.32	1.27	2.24
8	*trans* β-ocimene	1024	1030	6	0.44	0.47	3.36
9	γ-terpinene	1038	1043	5	0.33	0.40	9.24
10	α-thujone	1081	1088	7	19.01	19.07	0.17
11	β-thujone	1089	1095	6	4.40	4.04	4.25
12	camphor	1110	1117	7	14.02	14.34	1.13
13	borneol	1136	1142	6	2.12	1.86	6.65
14	bornyl acetate	1255	1262	7	0.89	0.85	2.07
15	β-caryophillene	1081	1088	7	5.79	4.82	9.11
16	α-humulene	1416	1418	2	8.74	7.70	6.35
17	ledene	1542	1548	6	9.80	8.43	7.55
18	caryophillene oxide	1552	1559	7	1.84	1.90	1.84
19	sclareol	1988	1994	6	6.79	8.09	8.75

471

472

Figure captions

Figure 1: Planar column compared to a one Euro cent coin.

Figure 2: Van Deemter plots of conventional (A) and chiral (B) planar and NB reference columns.

Figure 3: GC profiles (A) of chamomile e.o. obtained with the FFAP-EXT planar column 2 (solid line), compared to the reference (ref. 2) NB column (dashed line), and (B) of chamomile headspace sampled by SPME. Analysis conditions: temperature program: 50°C//15°C/min//190°C, flow rate: EOF (see Table 1). Peak identification: 1: farnesene, 2: germacrene D, 3: bisabolol oxide B, 4: bisabolone oxide B, 5: α-bisabolol, 6: chamazulene, 7: bisabolol oxide A, 8: spiroether.

Figure 4: GC profiles of "menthols" standard mixture, obtained with (A) FFAP-EXT planar column 3, and (B) Sil-5%-PH planar column 1. Analysis conditions: temperature program: 50°C//15°C/min//190°C for (A) and 50°C//2°C/min//190°C for (B), flow rate: EOF (see Table 1). Peak identification: 1: menthone, 2: isomenthone, 3: mentyl acetate, 4: neomenthol, 5: isomenthol, 6: menthol, 7: neoisomenthol.

Figure 5: GC profiles of peppermint e.o. obtained with (A) FFAP-EXT planar column 3, (B) Sil-5%-PH planar column 1, (C) with the reference Sil-5%-PH NB column (ref. 1). Analysis conditions: temperature program: 50°C//5°C/min//190°C for (A) and (C) and 50°C//2°C/min//190°C for (B), flow rate: EOF (see Table 1). Peak identification: 1: menthone, 2: isomenthone, 3: mentyl acetate, 4: neomenthol, 5: isomenthol, 6: menthol, 7: neoisomenthol, 8: α-pinene, 9: β-pinene, 10: 1,8-cineole, 11: limonene, 12: menthofuran, 13: terpinen-4-ol, 14: β-caryophillene, 15: germacrene D.

Figure 6: GC profiles of sage e.o. obtained with (A) the Sil-5%-PH planar column 1 and (B) with the reference Sil-5%-PH NB column (Ref. 1). Analysis conditions: temperature program: 50°C//5°C/min//190°C for (A) and 50°C//2.2°C/min//190°C (translated method) for (B), flow rate: EOF (see Table 1). Peak identification: 1: α-pinene, 2: camphene, 3: β-pinene, 4: β-mircene, 5: *p*-cymene, 6: 1,8-cineole, 7: limonene, 8: *trans* β-ocimene, 9: γ-terpinene, 10: α-thujione, 11: β-thujione, 12: camphor, 13: borneol 14: bornyl acetate,15: β-caryophillene, 16: α-humulene, 17: ledene,18: caryophillene oxide, 19: sclareol.

Figure 7: GC profiles of (A) chiral test obtained with the Et-β-CD planar column 5, and (B) with reference Et-β-CD NB column (ref. 3), (C) linalool derivatives standard mixture analyzed with the Et-β-CD planar column 5 (solid line) and with the reference Et-β-CD NB column (ref. 3) (dashed line), and (D) C6-C12 γ-lactones standard mixture obtained with the Et-β-CD planar column 5 (solid line) and with the reference Et-β-CD NB column (ref. 3) (dashed line). Analysis conditions:

temperature program: 50°C//2°C/min//190°C for (A) and (B) and 50°C//5°C/min//190°C for (C) and (D), flow rate: EOF (see Table 1). Peak identification: 1: limonene, 2: 2-octanol, 3: camphor, 4: isobornyl acetate, 5: linalyl acetate, 6: 2-methyl-3-(Z)-hexenyl butyrate, 7:menthol, 8: hydroxycitronellal, 9:γ-decalactone, 10:δ-decalactone; a: *(R)* enantiomer, b: *(S)* enantiomer, x and y: configuration not assigned.

Figure 8: GC profiles (A) of lavender e.o. and (B) of bergamot e.o obtained with no. 5 Et-β-CD planar column. Analysis conditions: temperature program: 50°C//5°C/min//190°C, flow rate: EOF (see Table 1). Peak identification: 1: β-pinene, 2: limonene, 3: camphor, 4: linalool, 5: linalyl acetate, 6: lavandulol acetate, 7: terpinen-4-ol, 8: lavandulol, 9: α-terpineol; a: *(R)* enantiomer, b: *(S)* enantiomer.

Figure 1

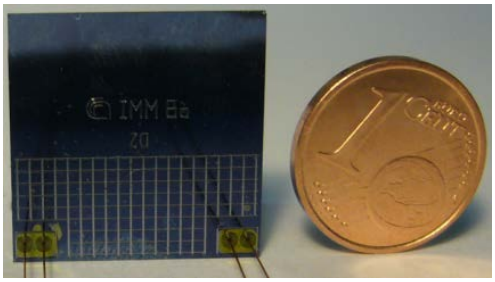
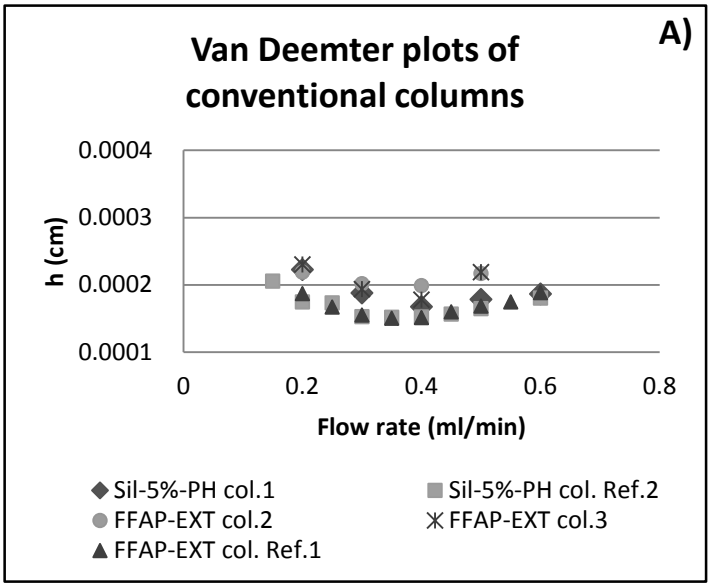


Figure 2


A)
Van Deemter plots of conventional columns
h (cm)
0.0004
0.0003
0.0002
0.0001
0
0.2
0.4
0.6
0.8
Flow rate (ml/min)
Sil-5%-PH col.1
Sil-5%-PH col. Ref.2
FFAP-EXT col.2
FFAP-EXT col.3
FFAP-EXT col. Ref.1

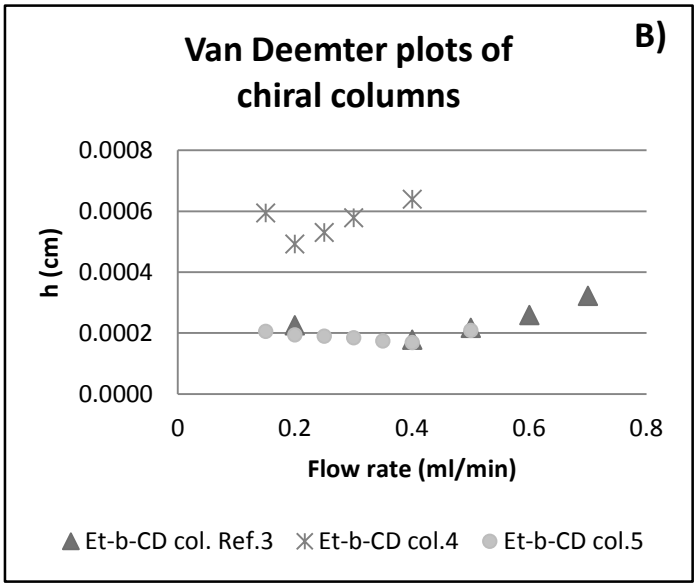
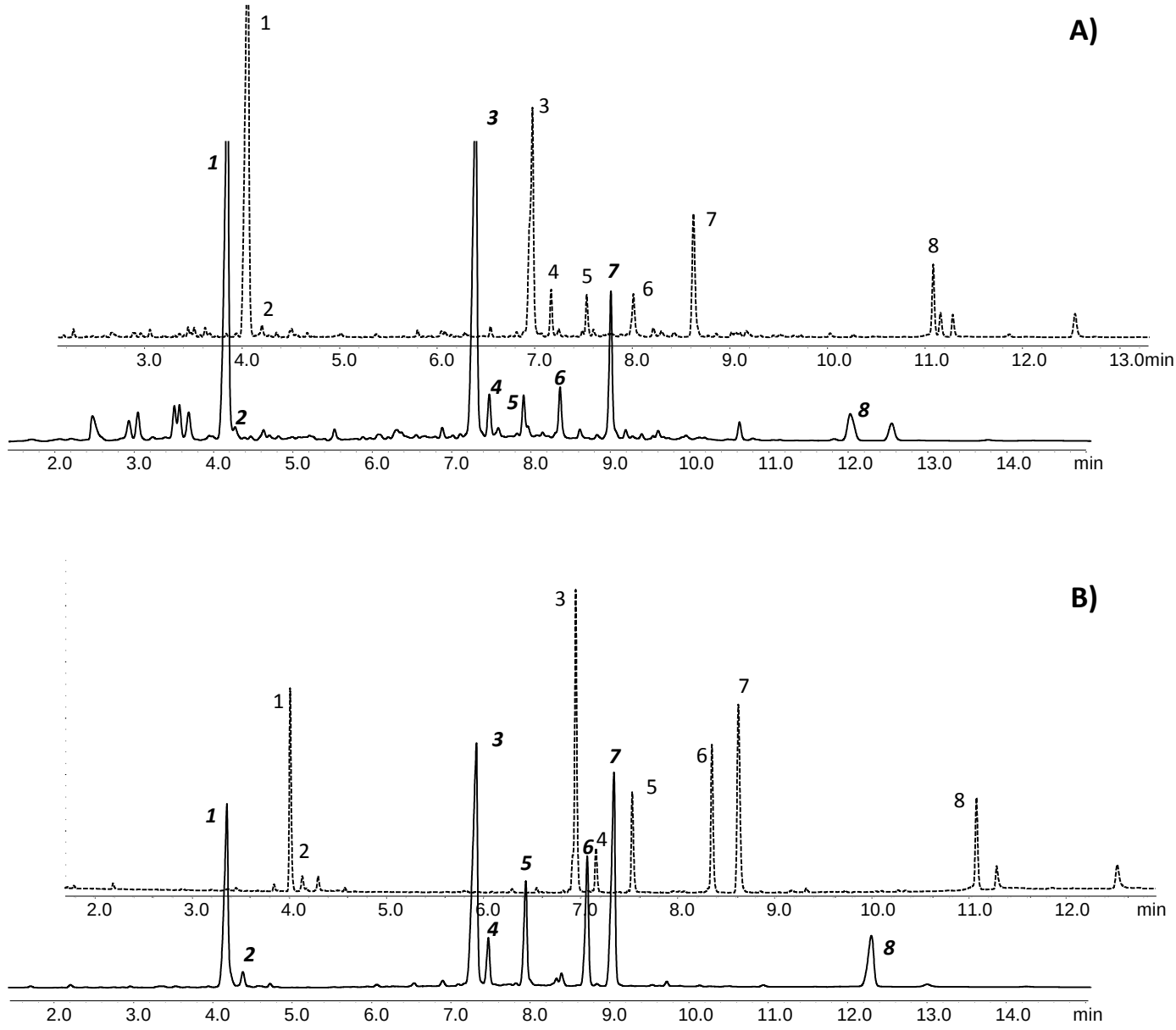

B)
Van Deemter plots of chiral columns
h (cm)
0.0008
0.0006
0.0004
0.0002
0.0000
0
0.2
0.4
0.6
0.8
Flow rate (ml/min)
Et-b-CD col. Ref.3
Et-b-CD col.4
Et-b-CD col.5

Figure 3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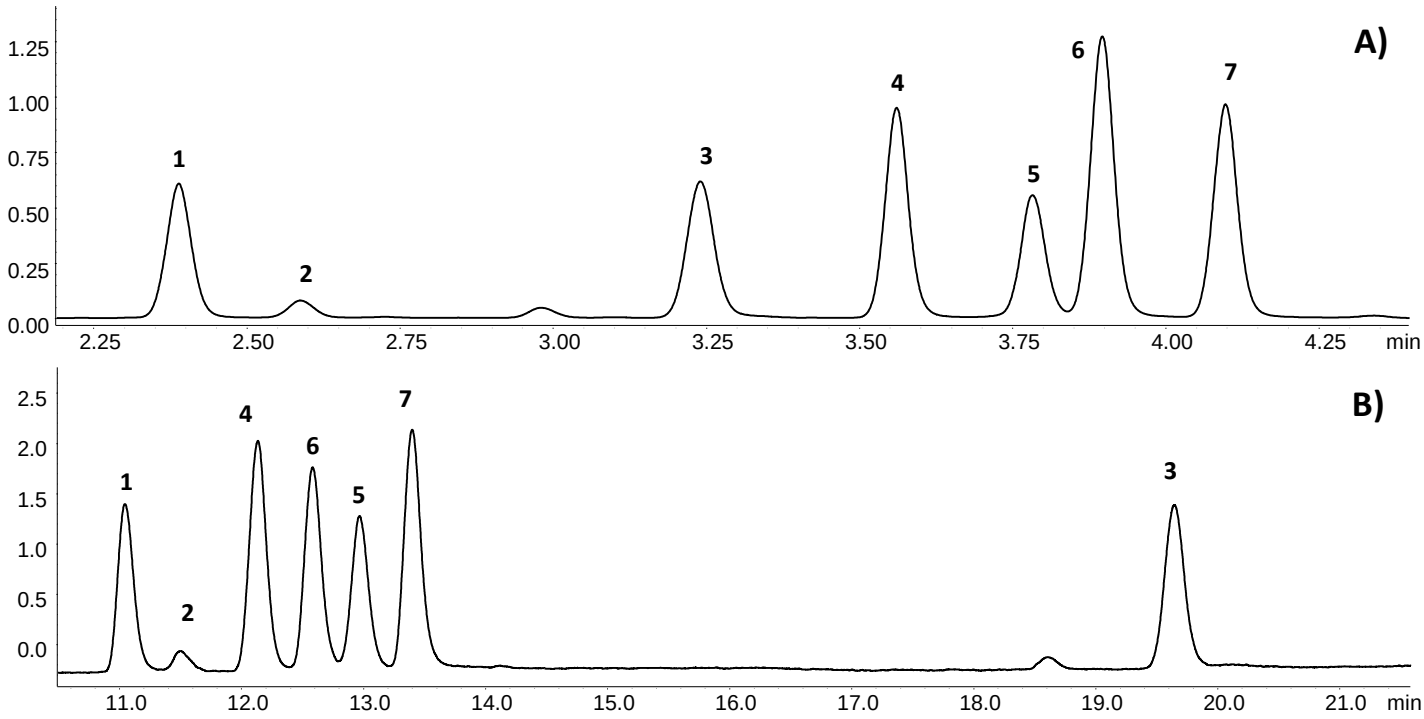
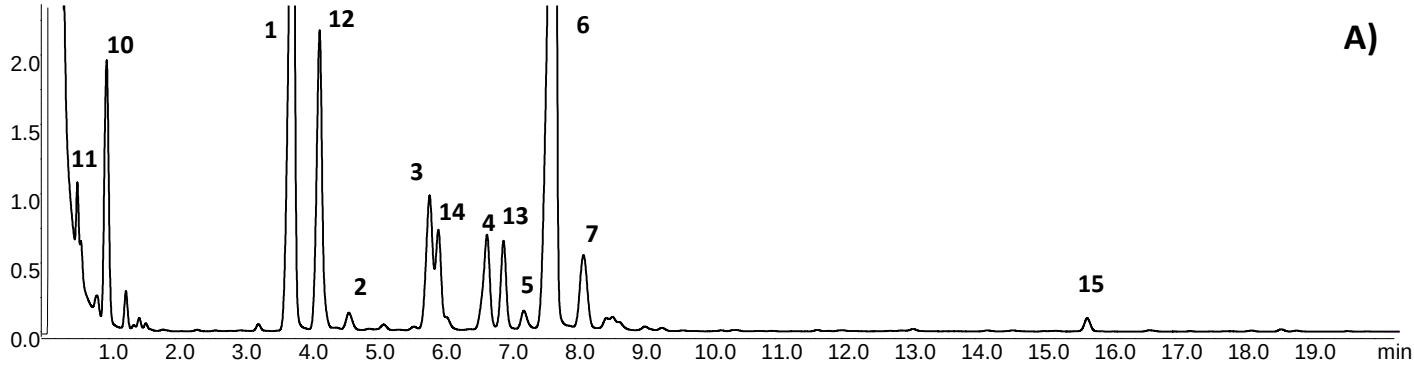
1
2
3
4
5
6
7
8
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0min
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
min
B)
1
2
3
4
5
6
7
8
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
min
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
min

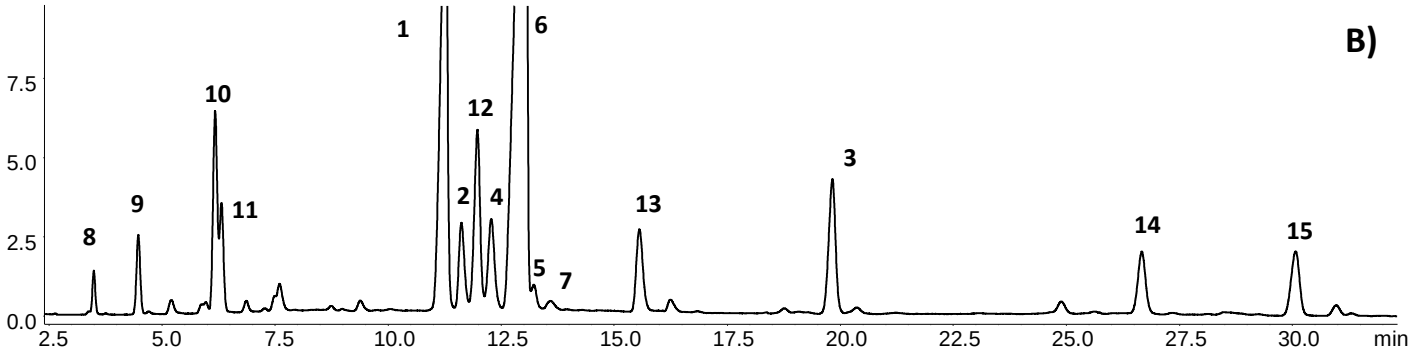
Figure 4


A)
1.25
1.00
0.75
0.50
0.25
0.00
1
2
3
4
5
6
7
2.25
2.50
2.75
3.00
3.25
3.50
3.75
4.00
4.25
min

B)
2.5
2.0
1.5
1.0
0.5
0.0
1
2
4
6
5
7
3
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
min

Figure 5


A)
1
12
10
6
11
3
14
4
13
7
2
5
15
2.0
1.5
1.0
0.5
0.0
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0 18.0 19.0 min


B)
1
6
10
12
3
9
2
4
11
13
14
15
8
5
7
7.5
5.0
2.5
0.0
2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 min

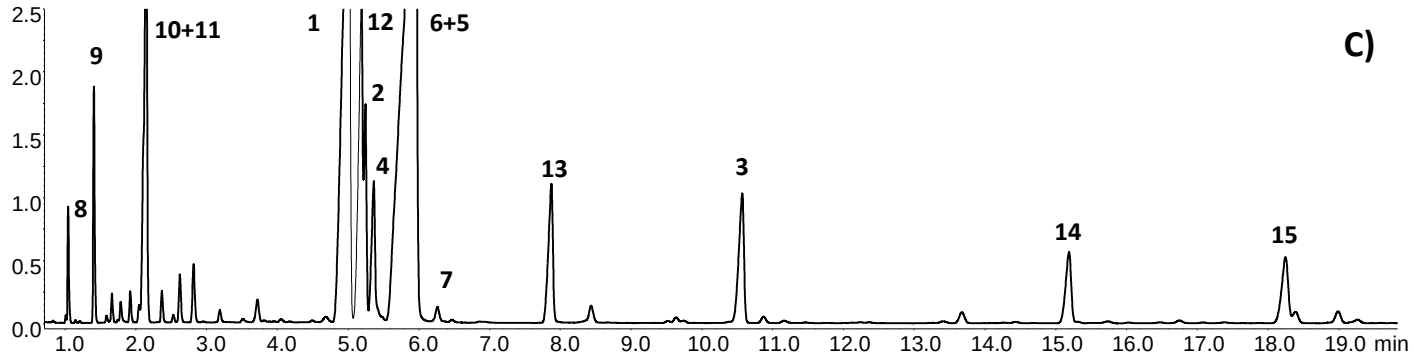
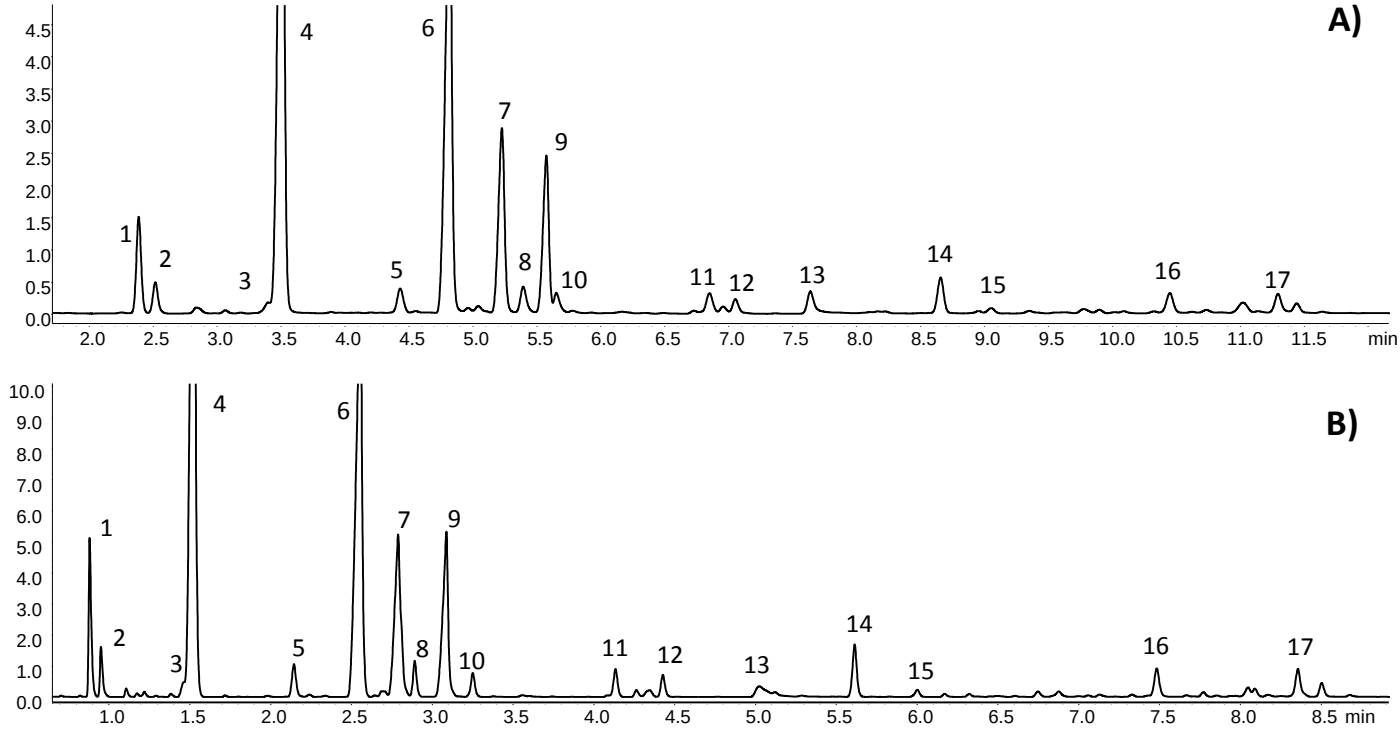

C)
10+11
1
12
6+5
9
2
4
13
3
8
14
15
7
2.5
2.0
1.5
1.0
0.5
0.0
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0 12.0 13.0 14.0 15.0 16.0 17.0 18.0 19.0 min

Figure 6


A)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 min

B)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 min

Figure 7

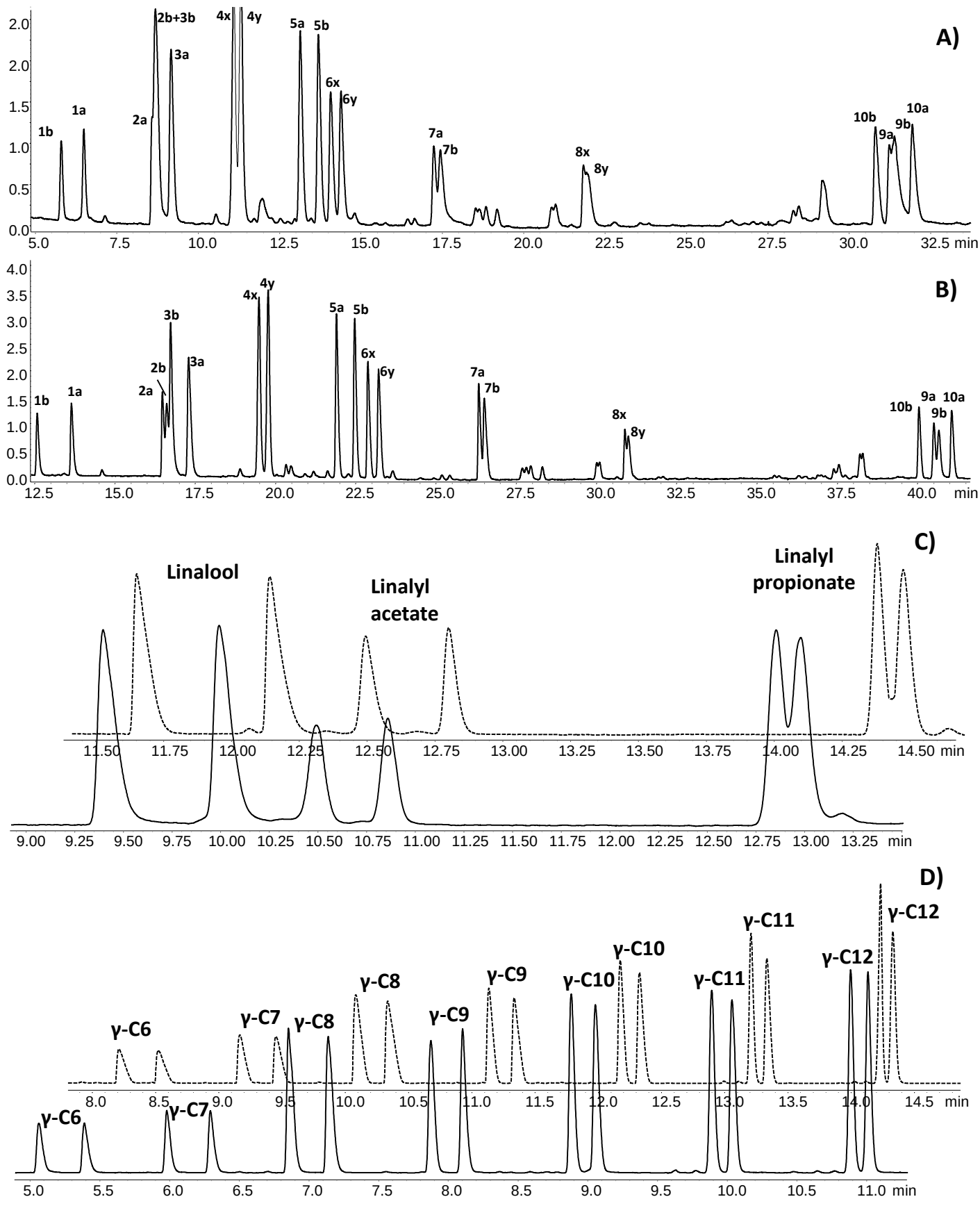
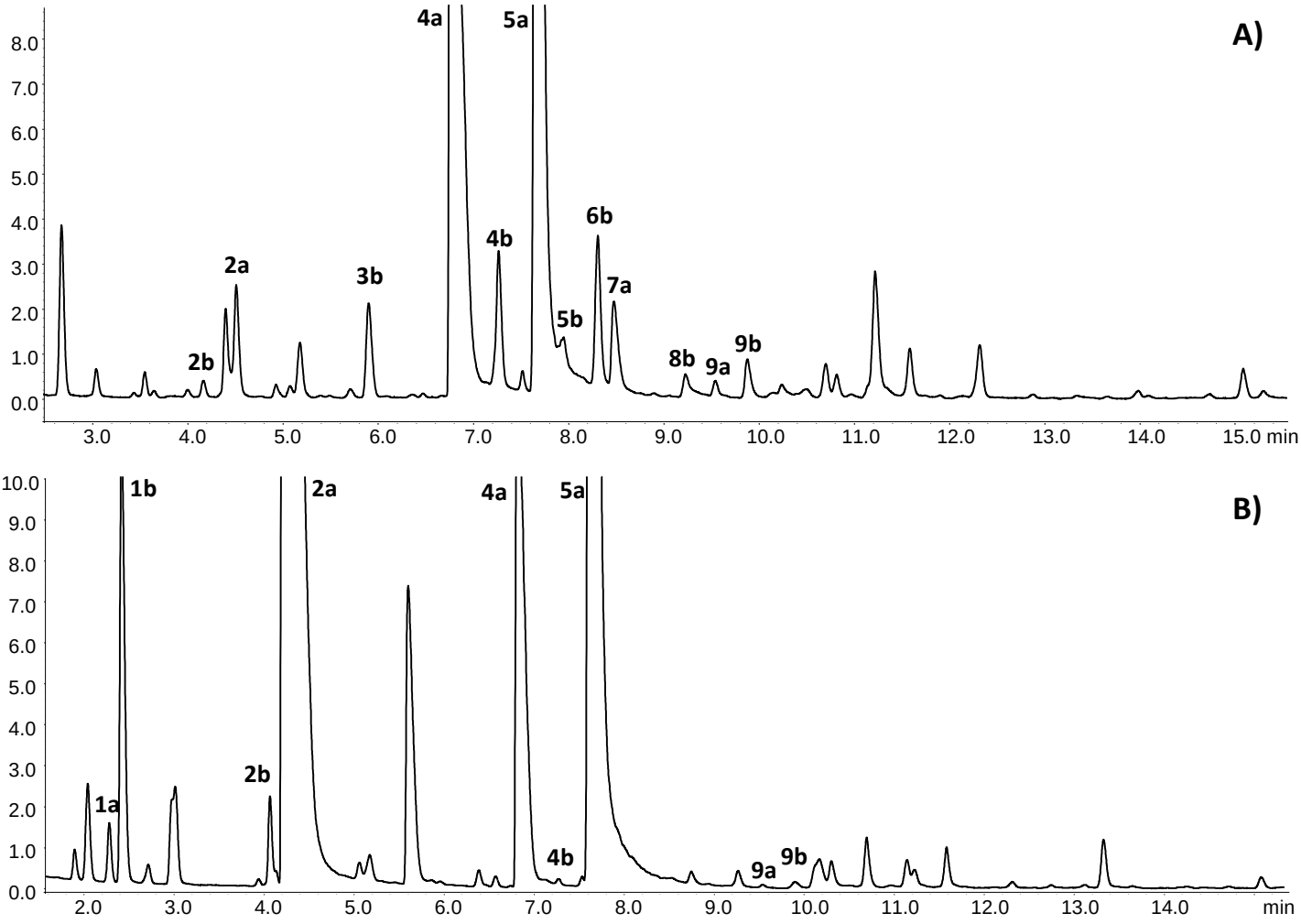

A)
1b
1a
2a
2b+3b
3a
4x
4y
5a
5b
6x
6y
7a
7b
8x
8y
10b
9a
9b
10a
min
B)
1b
1a
2a
2b
3b
3a
4x
4y
5a
5b
6x
6y
7a
7b
8x
8y
10b
9a
9b
10a
min
C)
Linalool
Linalyl acetate
Linalyl propionate
min
D)
γ-C6
γ-C7
γ-C8
γ-C9
γ-C10
γ-C11
γ-C12
min

Figure 8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4a
5a
6b
4b
2a
3b
7a
5b
2b
8b
9a
9b
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0 min
B)
1b
2a
4a
5a
2b
1a
4b
9a
9b
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
min

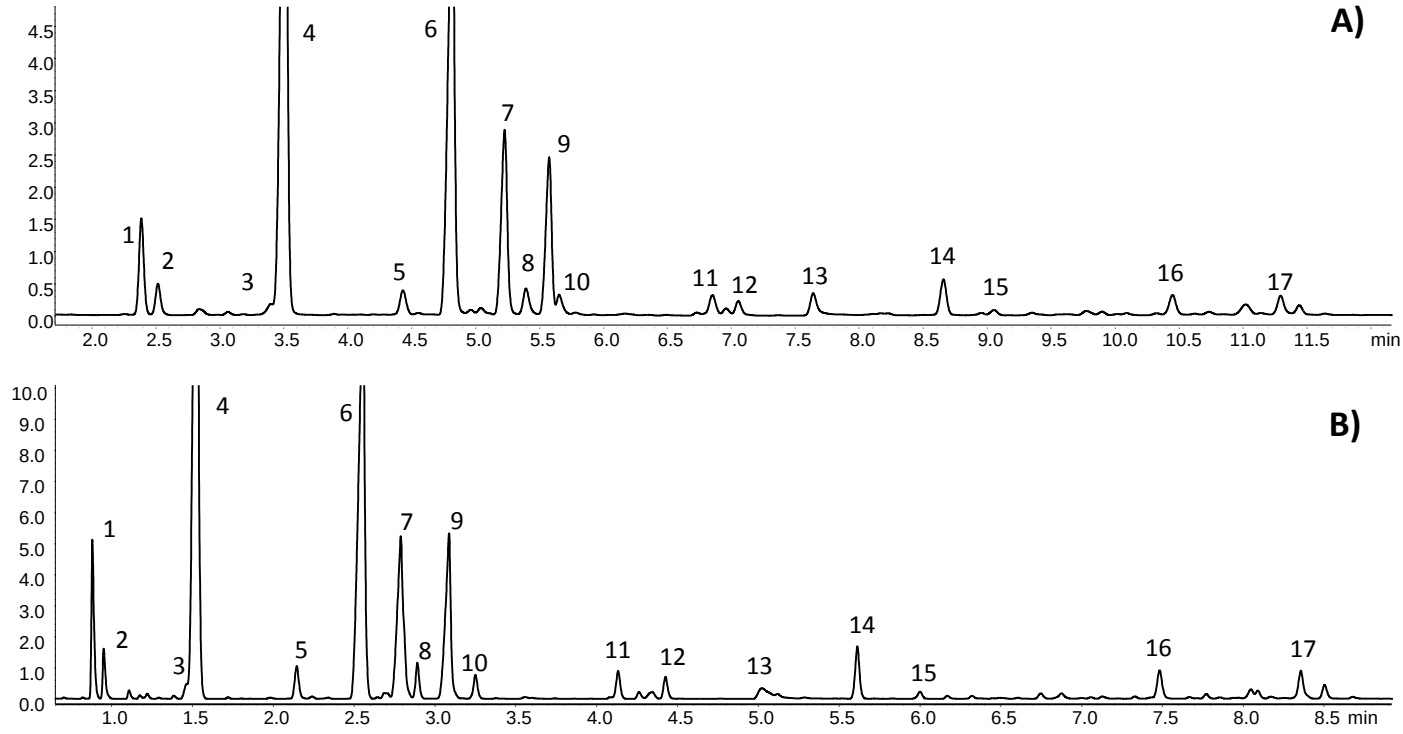

Figure 1SM: GC profiles of the rosemary e.o. obtained with (A) the Sil-5%-PH planar column 1 and (B) the reference Sil-5%-PH NB column (ref. 1). Analysis conditions: temperature program: 50°C//10°C/min//190°C, flow rate: EOF (see table 1). Peak identification: 1: α-pinene , 2: camphene, 3: limonene, 4: 1,8-cineol 2, 5: linalool, 6: camphor, 7: borneol, 8: terpinen-4-ol, 9: α-terpineol, 10: verbenone, 11: bornyl acetate, 12: carvacrol, 13: eugenol, 14: β-caryophillene, 15: α-humulene 16: δ-cadinene, 17: caryophillene oxide.

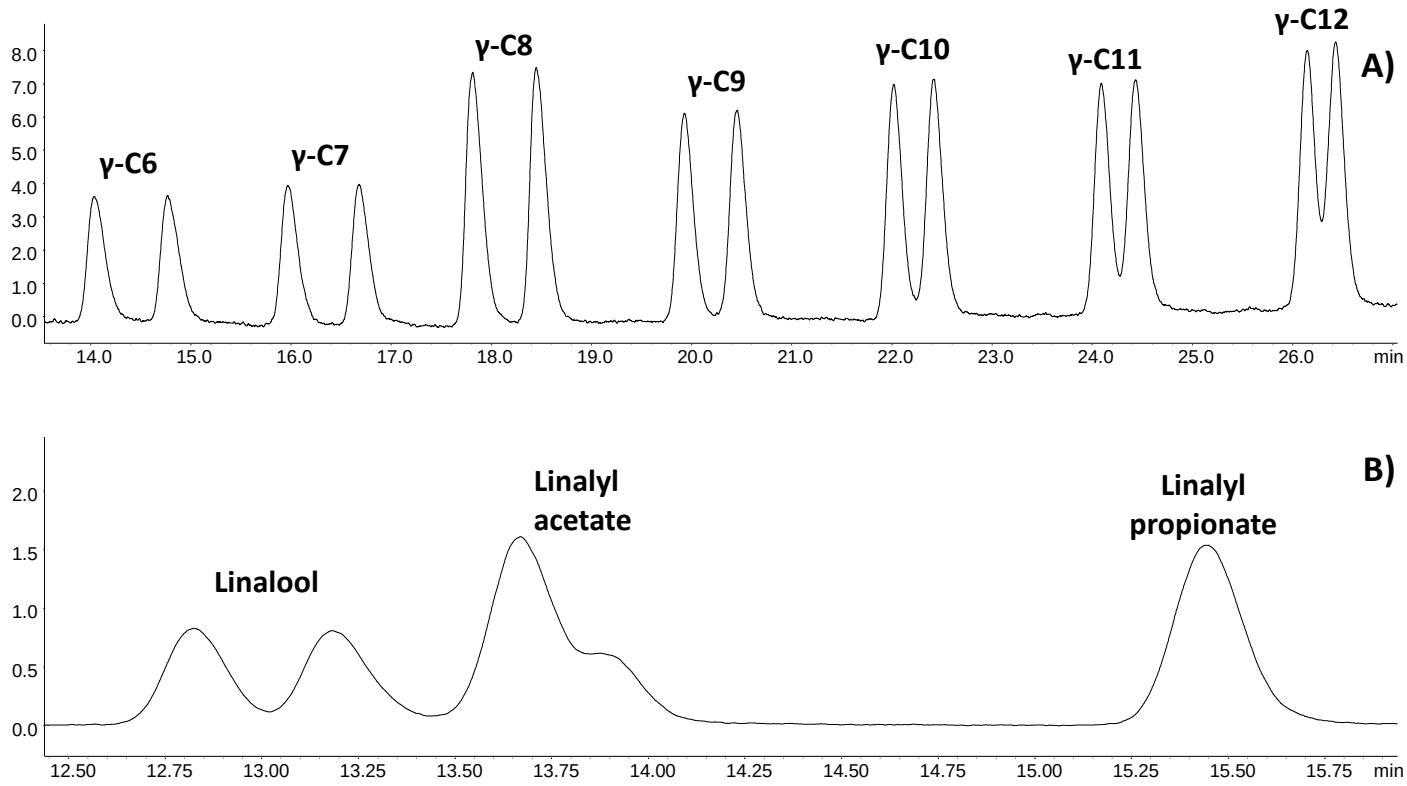

Figure 2SM: GC profiles of (A) the C6-C12 γ-lactones standard mixture and (B) of the linalools standard mixture, obtained with the Et-β-CD planar column 4. Analysis conditions: temperature program: 50°C//5°C/min//190°C, flow rate: EOF (see table 1)