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1 **Gas chromatography of essential oil: state of the art, recent advances and perspectives**

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13 **Abbreviations:** AEDA, aroma extract dilution analysis; CPS, chiral stationary phases; CD(s),
14 cyclodextrin derivative(s), d_c , column diameter; d_f , film thickness; Es-GC, enantioselective GC; EOs,
15 essential oils; FID, flame ionization detector; ILs, Ionic Liquids; MD, multidimensional; MDGC,
16 Multidimensional GC; NB, narrow bore; RRFs, relative response factors; SCC-GC, short capillary
17 columns with conventional inner diameters; t_0 : void time; TBDMS, tert-butyldimethylsilyl, UFM-
18 GC, Ultra-fast GC.

19

20 **Keywords:** conventional GC, enantioselective GC, essential oils, Fast GC, miniaturized GC

21

22

23 **Abstract**

24 This review is an overview of the recent advances of gas chromatography (GC) in essential oil (EO)
25 analysis; in particular, it focuses on both the new stationary phases and the advanced analytical
26 methods and instrumentations. A paragraph is dedicated to ionic liquids as GC stationary phases,
27 showing that, thanks to their peculiar selectivity, they can offer a complementary contribution to
28 conventional stationary phases for the analysis of complex essential oils and the separation of
29 critical pairs of components. Strategies to speed-up the analysis time, thus answering to the ever
30 increasing request for routine essential oils quality control, are also discussed. Last but not least, a
31 paragraph is dedicated to recent developments in column miniaturization in particular that based
32 on microelectromechanical-system technology (MEMS) in a perspective of developing micro-gas
33 chromatographic systems (μ -GC) to optimize the energy consumption as well as the
34 instrumentation dimensions. A number of applications in the essential oil field is also included.

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36

37

38 **1 Introduction**

39 Essential oils (EOs) are very complex mixtures of volatiles obtained by distillation processes (steam
40 distillation, dry distillation) or by cold expression, the latter used for species belonging to *Citrus*
41 genus [1, 2]. The definition reported in the European Pharmacopoeia clearly states that an EO is
42 the “Odorous product, usually of complex composition, obtained from a botanically defined plant
43 raw material by steam distillation, dry distillation, or a suitable mechanical process without
44 heating. Essential oils are usually separated from the aqueous phase by a physical process that
45 does not significantly affect their composition” [1]. Therefore, all products obtained with different
46 methods and techniques (i.e. solvent extraction, headspace sampling, and so on) cannot be
47 considered and called EOs.

48 The chemical composition of the EOs generally includes mono and sesquiterpenes and/or mono
49 and sesquiterpenoids, biosynthesised through the mevalonate/methyl erithrytol pathway, and
50 phenolic compounds (i.e. phenylpropanoids), deriving from shikimic acid pathway, respectively
51 [3].

52 The analysis of the EOs is generally required i) to evaluate their composition through the
53 characterisation of their chemical pattern (generally in terms of normalized relative abundance or
54 more seldom as absolute concentration of one or more markers) to be compared with the
55 Pharmacopoeia monographs or ISO norm, if available; ii) to evaluate the presence of an
56 adulteration (i.e. addition of cheaper EOs or synthetic “natural like” compounds or vegetable oils);
57 iii) to quantitate compounds that can be considered toxic or harmful for human use (e.g.,
58 suspected allergens [4]) and/or limited by regulatory authorities.

59 This review provides an overview of the state of the art and the recent advances on EO analysis
60 focusing the attention on the strategies suitable to characterize them correctly with the minimum

61 consumption of time and energy, i.e with a “green” analytical approach. The text covers the
62 literature concerning the GC analysis of EOs over the period 2000-2021.

63

64 **2 Conventional GC analysis**

65 Being the components of the EOs mainly volatiles (or semi-volatile), the technique of choice for
66 the analysis of the EOs is certainly gas chromatography (GC) [5] that is commonly coupled with a
67 flame ionization detector (FID) or a mass spectrometer (MS) with a single quadrupole, i.e. the
68 most common and cheap MS detectors for routine analysis, as analyser. The core of the
69 chromatographic system is represented by the column whose efficiency and selectivity depends
70 on the column characteristics and quality and on the stationary phase chemistry, respectively.
71 More in detail, the selectivity of a stationary phase is influenced by its polarity and solvation
72 properties [6]. In general, EO analysis is carried out with an apolar polysiloxane-based stationary
73 phases often in combination with moderately polar stationary phases based on polyethylene
74 glycol to overcome the component co-elutions and to obtain complementary standardized
75 chromatographic data [7, 8]. The most used apolar stationary phases in EOs routine analysis are
76 those based on methyl polysiloxanes (SE-30, OV-1, OV-101, DB-1, HP-1, PS-347.5 etc.) and methyl-
77 phenyl-polysiloxanes (SE-52, SE-54, DB-5, HP-5, PS-086 etc.), while the most applied moderately
78 polar phases are mainly based on different polyethyleneglycols (PEG-20M, CW-20M, DB-Wax,
79 etc.). Medium-polarity phases based on cyanopropyl-phenyl polysiloxane (i.e. OV-1701, DB-1701
80 and similar) are also used [9]. Stationary phases with different polarities are required since EOs are
81 complex mixtures often consisting of isomeric and/or analogous and homologous components
82 belonging to different chemical classes (alcohols, aldehydes, esters, ketones, oxides, ethers,
83 aromatics, etc.) with similar structural and physical properties (e.g. mono- and sesquiterpenoids)
84 not easy to separate simultaneously with a single GC stationary phases and to be distinguished by

85 their MS spectra. The adoption of suitable column set-ups consisting of stationary phases with
86 orthogonal selectivity is therefore necessary not only to enable the widest separation of EOs
87 components but also to enable the components identification by the simultaneous combination of
88 chromatographic data (linear retention indices on different stationary phases (I^T s or LRI)) and MS
89 data. In both cases, a fundamental role is played by the availability of in-house, literature and
90 commercial dedicated I^T s and MS libraries and database [9]. The complex composition of the EOs
91 justifies the constant search for new stationary phases characterized by different selectivity and at
92 the same time with good chromatographic properties (i.e. ionic liquids).

93

94 **3 High-speed GC in EOs analysis**

95 High-speed GC for routine analysis was introduced by Proot et al. in 1986 [10] with the aim to
96 reduce the analysis time, while keeping both separation and qualitative and quantitative results.
97 The use of high-speed GC in EO analysis gains an ever increasing attention because of the need to
98 increase laboratory throughput and to reduce the cost per analysis by reducing the time required
99 to obtain the results. Two strategies are mainly adopted to speed-up EO GC analyses. The first
100 one is based on the use of short capillary columns with conventional inner diameters (SCC-GC)
101 [11]. This approach is based on the observation that the efficiency of a capillary column for a given
102 separation is frequently much higher than necessary. As a consequence, the analysis time can be
103 shortened by rationally reducing efficiency (i.e. column length) to the minimum value that can
104 allow the baseline separation of the target analytes. SCC-GC can successfully be applied to routine
105 quali-quantitative analysis of medium-complexity samples (up to about 30-40 components) by
106 adopting short columns (5-10 m) together with a suitable temperature program. For instance, this
107 method has successfully been applied to the analysis of chamomile (*Matricaria chamomilla* L.) and
108 rosemary (*Rosmarinus officinalis* L.) EOs and the results demonstrated that short columns with

109 conventional inner diameter (d_c) can shorten analysis time of medium-to-low-complexity samples
110 [11]. The examples reported in the study showed that the analysis time can be shortened by
111 factors from five to ten while maintaining the baseline resolution of the components, without
112 affecting quantitative results.

113 The second, and most popular approach, is based on short columns with narrow d_c (0.1 mm or
114 less) (Fast-GC, F-GC) [12]. This approach not only increases analysis speed and analyte
115 detectability, because of peak sharpening, but also reduces carrier-gas consumption, as the
116 optimal flow rate for narrow-bore columns is considerably lower than that of columns with
117 conventional inner diameters. In the selection of the best strategy to increase the speed of
118 analysis, advantages and limits about the separation capability (peak separation and peak
119 capacity) of the two approaches must be considered. It is important to notice that the efficiency is
120 drastically reduced when SCC-GC is adopted while in F-GC, a suitable optimization of the column
121 geometry (column length and inner diameter) can keep similar if not better performance as that of
122 conventional GC. At the same time, the reduction of the column inner diameter in F-GC results in a
123 lower sample capacity (that can be critical for EO consisting of components in a wide range of
124 concentration) and in an increased back-pressure with a consequent more sophisticated
125 technological requirement for instrumentation. Another parameter to consider when attempting
126 to reduce the analysis time is the use of rapid temperature programming. This parameter
127 influences much more than others the analysis speed, but it mainly shows its positive effects with
128 short GC columns [13].

129 In the EO field, high-speed analyses in general require less than 10-15 minutes, and they are
130 carried out with columns of 5 to 15m length, with d_c between 0.1 and 0.25 mm, and temperature
131 programs from 20 to 60°C/min [14, 15]. In 2002, Klee and Blumberg introduced the method
132 translation approach [16] to facilitate the optimization of the F-GC conditions; this procedure

133 allows to convert automatically the parameters adopted for conventional columns to narrow-bore
134 columns via dedicated software [17]. This approach distinguishes the GC parameters into two
135 categories, i.e. translatable and non-translatable. Translatable parameters are column length,
136 inner diameter and film thickness, carrier gas and flow rate, heating rates, duration of
137 temperature plateau(s), and outlet pressure (i.e. for detectors working at ambient or reduced
138 pressure, such as MS. Non-translatable parameters are stationary phase chemistry, phase ratio,
139 and initial and final temperatures. Two methods are considered translatable one another if they
140 have identical non-translatable parameters and the same normalized temperature program (i.e.
141 the temperature rate divided by the void time, t_0) [16].

142 The use of F-GC for routine analysis of EOs is generally combined with mass spectrometers with
143 quadrupole as analysers (F-GC-qMS). F-GC-qMS is increasingly necessary in the EOs field in view of
144 the recent regulatory aspects, which have introduced stringent recommendations and that require
145 an ever-increasing number of accurate quantitative analyses in the routine controls (e.g.
146 suspected allergens in perfumes). The combination of F-GC with qMS must take into consideration
147 that the peak widths at half height are commonly below 0.5 s and that at least 10 point per peak
148 are required for an adequate peak re-construction thus resulting in a 50 Hz sampling frequency is
149 required in nearly all fast GC applications. This frequency is difficult to achieve even with the most
150 recent quadrupole analysers but it can be at least partially overcome by reducing the mass range
151 (300 amu), having most of the EO components molecular mass below this value.

152 Mondello et al. in 2004 [18] investigated the application of a F-GC to the analysis of five *Citrus* EOs,
153 bergamot (*Citrus limon* (L.) Osbeck), bitter and sweet orange (*Citrus × aurantium* L.), mandarin
154 (*Citrus reticulata* Blanco), and lemon (*Citrus limon* (L.) Osbeck). The F-GC method adopted required
155 the optimization of the experimental conditions (high inlet pressures, accelerated temperature
156 program rates, and split ratios) and, therefore, the adoption of a suitable instrumentation.

157 Although a very minor loss in peak resolution has been observed, the analytical results were
158 excellent, being the analysis time decreased to 3.3 min, with a speed gain of about 14 times when
159 compared to traditional GC analyses.

160 In this sense, in 2008, Rubiolo et al. [19] also investigated the possibility to apply a F-GC-qMS
161 method with narrow bore (NB) columns (10 m, 0.1 mm d_c , 0.10 μm d_f) to the analysis of a
162 peppermint (*Mentha × piperita* L.) EO, chosen as a model. The Authors evaluated the separation,
163 identification and quantitation performance of ten marker compounds characteristic of this EO, by
164 comparing the results to those obtained by a conventional GC approach. All the observed results
165 with F-GC were fully superimposable or better than those on conventional column, while achieving
166 a reduction of the analysis time by a factor of about ten (from about 35 to 3-4 min). Figure 1
167 reports the Peppermint EO TIC patterns resulting from a) conventional GC-qMS analysis, b) F-
168 GCqMS at 20° C/min and c) F-GC-qMS at 60°C/min[with permission from 19]. In the same year,
169 Poynter et al. [20] compared the results of Fast-GC analyses of parsley (*Petroselinum crispum*
170 (Mill.) Fuss) and fennel (*Foeniculum vulgare* Mill.) EOs with a NB capillary column (10 m, 0.1 mm
171 d_c , 0.10 μm d_f) with those obtained with a fast low-pressure gas chromatography–mass
172 spectrometry (GC–MS) with a 530 μm d_c column and a suitable capillary restrictor at the inlet of
173 the column. Between the two approaches, the low-pressure GC–MS was more successful for the
174 characterisation of the two investigated EOs. Although efficiency was sacrificed, the improved
175 sample capacity of the 530 μm d_c column leads to better peak intensities and thereby better mass
176 spectral library matching, thus providing highly satisfactory results. Tranchida et al. [21] also used
177 F GC in combination with rapid-scanning quadrupole mass spectrometry to analyse a basil
178 (*Ocimum basilicum* L.) EO. Analysis time was reduced from 25 min with conventional GC-MS to 5.3
179 min in F-GC. Resolution was altogether similar, with the same number of compounds reliably-
180 identified, proving the usefulness of F-GC in the field of EOs analysis.

181 In 2013, Tranchida et al. [22] also evaluated -GC in combination with a triple quadrupole mass
182 spectrometer (QqQ MS), enabling the possibility to operate by alternating full scan and multiple
183 reaction monitoring modalities (MRM) in the same analysis and in a high-speed manner. The study
184 involved the analysis of different EOs, namely mandarin (*C. reticulata* Blanco), lemon (*C. limon* (L.)
185 Osbeck), sweet orange (*C. × aurantium* L.), and bergamot (*C. limon* (L.)).

186 The EO analysis time can further be reduced by adopting 0.05 mm d_c columns. These columns
187 have found rare application in high-speed analysis probably because of the further reduction of
188 sample capacity and for a decreases the column lifetime due to the very thin stationary phase
189 thickness. However, 0.05 mm d_c columns can be applied to analyses of few minutes while
190 maintaining an acceptable column efficiency. For instance, in 2004 Mondello et al. [23] reported
191 the use of a 5 m, 0.05 mm $d_c \times 0.05 \mu\text{m } d_f$ for the analysis of lime (*Citrus aurantiifolia* (Christm.)
192 Swingle) EO. Because of the very high efficiency of the column (about 20.000 plates per meter),
193 0.05 μm capillary columns with fast temperature programming allowed EO analysis in less than 90
194 s. High GC resolution was maintained, and quantitation of key components for quality assurance
195 purposes is well achievable using FID as detector, without a peak de-convolution as previously
196 reported [24]. Figure 2 shows the Fast GC analysis of lime EO using a 5 m, 0.05 mm $d_c \times 0.05 \mu\text{m } d_f$
197 capillary column and fast temperature programming [with permission from 23].

198 Ultra-fast module GC (UFM-GC) separations with analysis times lower than 1 minute can also be
199 achieved by adopting temperature programming rates up to 20°C/s although it requires the
200 adoption of a direct resistive heating of the capillary column and a coupling with very high
201 frequency detection systems such as Time of Flight (ToF-MS) or FID [25-26]. This approach has
202 been successfully adopted for the analysis of a series of EOs characterized by different complexity.
203 Bicchi et al. [25] applied UFM-GC to routine analysis of chamomile (*M. chamomilla* L.), peppermint
204 (*M. x piperita* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) EOs and

205 compared the results with direct resistively heated columns to those of conventional GC (C-GC)
206 with conventional d_c columns (0.25 mm) of different lengths (5 and 25 m long), and to those of F-
207 GC with NB columns (0.1 mm d_c and 5 m long). This study showed that UFM-GC is effective both
208 for qualitative and quantitative analysis of EOs of different compositions enabling to reduce
209 drastically the analysis time; however, as expected, the very high column heating rates produce
210 changes in selectivity compared to C-GC [27]. In 2009, the same approach was adopted by Heuskin
211 et al. [28] to characterise the chemical composition of *M. chamomilla* L. and *Nepeta cataria* L. EOs.

212

213 **4 Enantioselective gas chromatography**

214 Biosynthetic pathways in plants are often stereochemically-driven and, generally, they lead to
215 chiral compounds, in some cases, also in an enantiomeric excess. Enantiomeric composition
216 determination is therefore fundamental to obtain a full phytochemical characterization of a
217 natural matrix and to determine its biological activity. This consideration is particularly true for
218 EOs, where enantiomeric composition enables (a) to define the geographical origin of a sample,
219 (b) to detect adulteration in “natural” samples, and (c) to relate a chemical structure to its
220 organoleptic properties. The absolute configuration of a natural compound is not only related to
221 its biosynthesis, but also to its biological activities as confirmed by the organoleptic properties of
222 some chiral odorants whose enantiomer are characterized by completely different odour. The first
223 detected example was β -citronellol reported by Rienacher and Ohloff in 1961 [29]: the (+)-
224 enantiomer has a typical citronella odour, while the (-)-enantiomer is evocative of a geranium
225 smell. Other highly representative EO components show different organoleptic features between
226 the two enantiomers including limonene ((*R*): orange like, (*S*): turpentine notes), α -terpineol ((*R*):
227 flowery sweet, (*S*): tarry, cold pipe), carvone ((*R*): herbaceous, dill seeds, (*S*): herbaceous,
228 spearmint), and α -phellandrene ((*R*): citrus odour, (*S*): weed/dill like) [30, 32]. The most common

229 and practical approach to separate the enantiomers of the volatile components of a natural matrix
230 is by GC with columns coated with a chiral selectors able to recognize (i.e. to separate) its
231 enantiomers, and therefore to determine its enantiomeric composition. The first chiral stationary
232 phases (CSP) based on N-trifluoroacetyl-L-isoleucine lauryl ester was introduced in 1966 by Gil-Av
233 et al. [32, 33] followed by several other chiral selectors. The CSPs most adopted in the EO analysis
234 are those based on cyclodextrin derivatives (CDs) thanks to their high enantioselectivity and their
235 wide range of applications. Cyclodextrins, also known as cycloglucanes, cycloamiloses or
236 cyclomaltoligos, are a homologous series of non-reducing cyclic oligosaccharides made up of six-
237 to-twelve (β)-D-glucopyranose units linked by α -1-4-glycoside bonds, deriving from the enzymatic
238 degradation of the starch by cyclodextrin glycosyltransferase. The best known and most widely
239 used CDs are those with six (α -CD), seven (β -CD) or eight (γ -CD) sugar units.

240 The first CD-based SP for enantioselective GC (Es-GC) made up of a mixture of native α -
241 cyclodextrin in formamide was introduced by Koscielski and Sibilska [34] in 1983 to separate the
242 enantiomers of Δ -3-carene, α - and β -pinene, and some hydrogenated derivatives. The native
243 cyclodextrins were soon replaced by per-alkylated cyclodextrins that showed drastically better
244 chromatographic and enantioselective properties [35, 36]. In the 90's an important innovations
245 werer introduced by Blum and Aichholz [37] and by Mosandl's research group [38] that proposed
246 the introduction of a bulky substituents (in particular the *tert*-butyldimethylsilyl, TBDMS) at the
247 primary C6-hydroxyl groups of the sugar units and smaller substituents at the C2- and C3-
248 secondary hydroxyl groups, the latter being responsible for enantioselectivity, to extend the range
249 of applications of CD derivatives. Despite the interest for CD-based CSPs, over the last ten years, a
250 few new CD derivatives with higher, wider or "new" enantioselectivity have been introduced. In
251 2010, Bicchi et al. [39] have introduced "fully asymmetrically substituted derivatives"
252 characterized by different substituents at the free hydroxyl moieties of the cyclodextrin sugar

253 units (2-*O*-methyl-3-*O*-ethyl- or 2-*O*-ethyl-3-*O*-methyl-6-*O*-TBDMS - β -derivatives, MeEt-TBDMS CD
254 and EtMe-TBDMS CD respectively). They evaluated the chromatographic properties and
255 enantioselectivity of the new asymmetrical derivatives and compared them to those of the
256 corresponding symmetrically substituted (2-*O*-methyl-3-*O*-methyl- and 2-*O*-ethyl-3-*O*-ethyl-6-*O*-
257 TBDMS - β -derivatives, MeMe-TBDMS CD and EtEt-TBDMS CD respectively) by analysing by
258 analysing a set of EOs of different complexity, i.e. bergamot (*C. limon* (L.) Osbeck), lavender
259 (*Lavandula angustifolia* Mill.), lemon (*C. limon* (L.) Osbeck), bitter and sweet orange (*C. ×*
260 *aurantium* L.), peppermint (*M. × piperita* L.), rosemary (*R. officinalis* L.) and sage (*S. officinalis* L.).
261 Figure 3 reports the enantioselective GC-MS profiles of bergamot EO analysed with columns
262 coated respectively with MeMe-TBDMS CD (a), MeEt-TBDMS CD (b), EtMe-TBDMS CD (c), EtEt-
263 TBDMS CD (d), all dissolved in PS-086 [with permission from 39]. The results clearly demonstrated
264 that asymmetrically substituted methyl/ethyl CDs increased the enantioselectivity compared to
265 the corresponding symmetrical methyl or ethyl derivatives, in terms of both enantiomer resolution
266 and number of chiral compounds separated. In particular, for bergamot EO EtMe-TBDMS CD
267 separated the enantiomers of all seven chiral components simultaneously and with resolutions
268 above 1.5 (i.e. α -pinene, β -pinene, sabinene, limonene, linalool, linalyl acetate and α -terpineol).
269 Conversely, symmetrical CDs showed an insufficient separation of linalyl acetate and α -pinene
270 enantiomers and a co-elution of (*R*)-sabinene and β -myrcene.
271 An important limit conditioning the application of Es-GC is the long analysis time conventionally
272 required, because of the difference in the energy of association between each enantiomer and the
273 CD chiral selectors. At the same time, enantiomers separation is regulated by thermodynamics (i.e.
274 by temperature), thus requiring a very high chromatographic efficiency and, therefore, long
275 columns and low temperature rates are commonly adopted. Despite these limitations, F-GC can
276 also be applied to Es-GC by reducing length column, inner diameter and flow rate, i.e. NB columns

277 [19, 33]. In 2008, Bicchi et al. [40] compared a conventional inner diameter CSP-based column
278 coated with 30% of 2-*O*-ethyl-3-*O*-ethyl-6-*O*-TBDMS- β -cyclodextrin to a conventional 0.25mm d_c
279 short column and to different length NB columns in analysing both bergamot and lavender EOs.
280 NB columns provided shorter analysis time and resolutions than those of conventional d_c CSP
281 columns, allowing the most effective compromise between separation efficiency and analysis
282 time. In 2010, the same research group [41] developed an optimization approach for routine Fast
283 Es-GC analyses of EOs, using lavender EO as model sample. They showed that optimization
284 allowed the reduction of the analysis time by a factor of at least three using NB columns in
285 comparison with conventional not-optimized GC and by a factor of two adopting optimized
286 conditions with conventional inner diameter columns, while maintaining the same separation of
287 chiral markers and efficiency.

288 In the last decade the enantiomeric analysis has gained more and more importance in view of a
289 complete characterization of EOs in order to assess the authenticity and genuineness, as
290 evidenced by the number of publications in the literature [among others 42-50].

291 In any case, the authors also periodically reviewed in details the applications concerning the chiral
292 recognition of chiral components in essential oils reported in the literature [33, 51, 52].

293 EsGC may also be combined with multidimensional GC, enabling an in depth recognition of
294 diagnostic chiral components for EOs authentication (see below) [53, 55].

295

296 **5 Multidimensional GC in EO analysis**

297 In 1987, Giddings defined a multidimensional (MD) separation [56] as: "... an orthogonal two
298 column separation, with complete transfer of solute from the separation system 1 (column 1) to
299 the separation system 2 (column 2), such that the separation performance from each system
300 (column) is preserved". Two-dimensional comprehensive GC (GC \times GC) introduced by Liu et al. [57],

301 is the most recent and powerful GC technique now available perfectly meeting the above
302 definition. In GC×GC, each analyte eluting from the first column is on-line and automatically
303 trapped, refocused, and re-injected into the second column, by a thermal, flow or valve-based
304 focusing device (modulator).

305 To the best of the authors' knowledge, the first applicatin of GCxGC to EO analysis was on vetiver
306 (*Chrysopogon zizanioides* (L.) Roberty) EO due to Marriot's group [58]; since then, several GC×GC
307 applications in the EOs analysis have been reported in the literature [among others 55, 59-70],
308 showing the great potentiality of this technique, in particular when EOs are characterized by
309 hundreds of components (i.e. *C. zizanioides* (L.) Roberty) or when they contain components with
310 very similar structure and therefore difficult to separate in a single run (e.g. sandalwood, *Santalum*
311 *album* L.), but decisive to define product quality and authenticity or to characterize a species.

312 In 2013, Filippi et al. profiled and quantified vetiver EO sesquiterpenoids [71]. Vetiver EO is
313 considered to be one of the most complex EOs, being its conventional GC analysis normally
314 characterized by a great number of coelutions, thus not sufficient as a routine tool for the
315 accurate qualitative and quantitative analysis of its EO constituents. This study applied a GC × GC-
316 FID/MS platform to separate and to quantitate efficiently vetiver EO constituents. This approach
317 allowed i) the identification of 135 constituents in four different samples of vetiver EO and ii), for
318 the first time, the reliable quantitation of all the identified constituents by means of internal
319 calibration.

320 In 2015, Sgorbini et al. [62] applied a GC×GC platform combined with a simultaneous dual
321 detection (i.e. FID and MS, namely GC×2GC-MS/FID) for the quantitative profiling of two medium
322 complexity EOs, i.e. mint (*M. × piperita*, *M. arvensis*, *M. × gentilis*, *M. spicata*) and lavender (*L.*
323 *angustifolia* and *L. angustifolia* × *L. latifolia*) species. The study aimed at evaluating the method
324 accuracy and the quantitation reliability. Quantitative determination of all the components was

325 performed by using the predicted relative response factors (RRFs) based on combustion enthalpies
326 and molecular structure [71-73]. The results showed that the applied GC×2GC-MS/FID platform is
327 highly reliable in terms of linearity, precision and quantitation accuracy. Moreover, the accuracy of
328 predicted RRFs supports their application in the quantitative analyses of markers in EOs, making
329 this approach of particular interest in those situations for which reference standard are not (easily)
330 available.

331 In GC×GC chiral recognition, the column coated with a chiral stationary phase must be installed in
332 the first dimension, because of the high efficiency required for effective enantiomer separations
333 (74, 75).

334 In 2015, Wong et al. [55] showed the potential of fast multiple heart-cut enantioselective
335 multidimensional gas chromatography (GC–EsGC) and enantioselective comprehensive two-
336 dimensional gas chromatography (ESGC×GC), to perform the enantiomeric analysis of three key
337 chiral monoterpenes (limonene, terpinen-4-ol and α -terpineol) in tea tree (*Melaleuca alternifolia*
338 (Maiden & Betche) Cheel) oil (TTO). In particular, the authors evaluated and discussed the
339 suitability of using these two enantioselective multidimensional approaches for the routine
340 assessment of chiral monoterpenes in TTO by analysing a representative number of pure (genuine)
341 Australian TTOs. The results were compared (using principle component analysis) with commercial
342 TTOs obtained from different continents. The results are an effective and useful reference for
343 setting an international standard based on chiral composition for TTO producers, suppliers,
344 manufacturers and consumers. The proposed method offered distinct advantages over
345 conventional enantioselective GC, especially in terms of analysis times and selectivity and can be
346 used as a reliable platform for authenticity control of TTO. Figure 4 reports the EsGC × GC–FID
347 contour plots of a TTO sample obtained using a ¹D MEGA-DEX DET-Beta phase and (A)

348 SUPELCOWAX®10 2D column, (B) SLB-IL59 2D column, (C) SLB-IL61 2D column, and (D) BPX5 2D
349 column [with permission from 55].

350 Very recently, Gabetti et al. [61] applied a GC×GC-ToF MS platform combined with an off-line 1D-
351 GC-O-AEDA (aroma extract dilution analysis) to study both fingerprinting and profiling of
352 peppermint (*M. × piperita* L.) EOs of different varieties and geographical origins. Most peppermint
353 EO nowadays are from United States (US), although Italy has maintained a high quality commercial
354 production localized in Piedmont (Northwest Italy). Piemontese peppermint EO is produced from a
355 single mint variety, i.e., *M. × piperita* L. var. Italo-Mitcham, and is appreciated for its freshness and
356 long-lasting sweetness. The study aimed at defining strategies for discrimination of high- quality
357 products by combining analytical data with sensory screening. Chromatographic fingerprinting by
358 GC ×GC-ToF MS resulted to be a powerful and reliable approach to define the characteristic
359 chemical signature of peppermint EOs and to identify and quantify trace components (mint
360 lactones) able to discriminate between Piemontese and US origins of peppermint EOs.

361

362 **6 Ionic liquids (ILs) as gas chromatographic stationary phases for EO analysis**

363 Ionic Liquids (ILs) are among the most largely investigated new materials for use as GC stationary
364 phases [76]. In the last twenty years, ILs have aroused great interest as new stationary phases for
365 GC. They have opened new possibilities thanks to their unusual and outstanding selectivity,
366 completely different from that of the conventional stationary phases, i.e. PDMS and PEG used in
367 this field. ILs-based stationary phase are characterized by a higher polarity than conventional SPs,
368 while maintaining the same chromatographic performance and similar (or higher) operative
369 temperatures. These features make IL-based stationary phases of great interest also in the EO
370 field.

371 ILs are salts consisting of one or more organic cation (i.e. mono or poly-cationic ILs, respectively)
372 containing phosphorous or nitrogen (usually imidazolium, phosphonium, pyridinium,
373 pyrrolidinium) cation and by one or more inorganic or organic anions [77-80]. They are
374 characterized by a melting point at or below 100°C, low vapour pressure, high thermal stability
375 (over 300°C) and different conductivity, viscosity and solvent miscibility [81]. Starting from 2008,
376 Supelco has introduced into the market several commercial ILs-based stationary phases (e.g. SLB-
377 IL59, SLB-IL60) and the studies for new derivatives as SP are still in progress.

378 The first application of ILs as stationary phase in the plant field dates back to 2007 by Qi et al. [82]
379 who applied a geminal dicationic IL (1,9-di(3-vinylimidazolium) nonanebis
380 [(trifluoromethyl)sulfonyl]imidate) for the analysis of cinnamon (*Cinnamomum verum* J.Presl),
381 fennel (*F. vulgare* Mill.) and nutmeg (*Myristica fragrans* Houtt.) EOs. Ragonese et al. [83] in 2011
382 investigated the use of a commercial IL-based column (SLB-IL59) in the analysis of a lemon (*C.*
383 *limon* (L.) Osbeck) EO *versus* two conventional stationary phase (i.e. polyphenylsiloxane and PEG).
384 In this study, the IL-based stationary phase showed polarity comparable to that of PEG columns
385 but higher thermal stability and different selectivity; in addition, the IL column gave better results
386 than conventional SP in terms of quantitative determination, while maintaining a stability of
387 retention indices comparable to that of apolar columns. In 2012, Cagliero et al. [84] carried out a
388 systematic evaluation of different commercially available IL-based SPs for the analysis of two EOs
389 characterized by a different chemical composition and complexity, i.e. cornmint (*M. arvensis*) and
390 vetiver (*C. zizanioides* (L.) Roberty). The study clearly showed that ILs-based SPs are extremely
391 useful for EOs analysis thanks to their uncommon selectivity to be leveraged when a separation on
392 functional group (as happens in EOs) is required. The study highlighted also the need for an
393 improvement of the inertness of ILs-based SPs to decrease their activity toward polar analytes. In
394 2017, the same authors [85] investigated a series of highly inert ILs-based SPs introduced in 2016

395 by Supelco, comparing their performance to those of conventional SPs and to those of the first
396 generation of ILs. They applied the second generation of the ILs-based columns to the analysis of
397 chamomile (*M. chamomilla* L.) and sandalwood (*S. spicatum* (R. Br.) A. DC) EOs and demonstrated
398 the successful application of these new SPs in the routine quali-quantitative characterization for
399 the EO quality control. Figure 5 reports the GC-FID patterns of sandalwood (*S. spicatum* (R. Br.) A.
400 DC) EO with a) OV1, b) PEG-20M, e) IL60, d) IL60i (optimized method) columns [with permission
401 from 85].

402 An important feature of ILs is the possibility to modulate their selectivity by modifying their
403 chemical composition thus enabling the establishment of different solvation interactions. In 2019,
404 Mazzucotelli et al. [86] studied in depth the chromatographic performance and the selectivity of
405 two phosphonium cation-based ILs, trihexyl(tetradecyl)phosphonium ($[P_{66614}^+]$), combined with
406 two different anions, i.e. chloride $[Cl^-]$ and bis[(trifluoromethyl)sulfonyl]imide $[NTf_2^-]$. They chose
407 sage (*S. officinalis*) and vetiver (*C. zizanioides*) EOs as examples of samples with different
408 complexity. The study proved their very peculiar selectivity: i) $[P_{66614}^+][NTf_2^-]$ was characterized by
409 a relatively short retention and a selectivity strictly related to the analytes' polarity and volatility,
410 and ii), $[P_{66614}^+][Cl^-]$ presented a relatively high retention and a selectivity based on the functional
411 groups of the analytes under investigation. Figure 6 shows GC-MS profile of sage (*Salvia officinalis*
412 L.) EO analyzed with $[P_{66614}^+][Cl^-]$ and $[P_{66614}^+][NTf_2^-]$ 5 m test columns [with permission from 86].
413 The $[P_{66614}^+][Cl^-]$ pattern shows a very clear separation between the components in agreement
414 with their organic function groups and number of carbon atoms, for example, (in order of elution)
415 monoterpenoids (C10) including hydrocarbons and 1,8-cineole, ketones, esters, sesquiterpene
416 (C15) hydrocarbons, and monoterpene alcohols. On the other hand, the $[P_{66614}^+][NTf_2^-]$ pattern
417 clearly discriminates between hydrocarbons and oxygenated monoterpenoids, the latter group of
418 peaks also incorporating sesquiterpene hydrocarbons. The same authors also applied $[P_{66614}^+][Cl^-]$

419 columns to the analysis of oregano (*Origanum vulgare* L.), peppermint (*M. x piperita* L.),
420 sandalwood (*S. album* L., *S. yasi* Seem), thyme (*T. vulgaris*) and frankincenses (*Boswellia socotrana*
421 Bolf.f.) EOs [87]. In particular, this study evaluated the optimal operative conditions for the
422 investigated [P₆₆₆₁₄⁺][Cl⁻] SP and showed that its peculiar and unique selectivity enables the
423 baseline separation of all compounds in sandalwood EO in a single run after optimizing column
424 characteristics and operative conditions.

425 ILs were also successfully applied to MDGC, in particular comprehensive two-dimensional GC
426 (GC×GC). In 2013, Tranchida et al. [88] used SLB-IL60 as second dimension in GC×GC to perform
427 both targeted and untargeted analyses on mandarin (*C. reticulata* Blanco) EO and to quantify five
428 pesticides in spearmint (*M. spicata*) EOs. As mentioned above, in 2015, Wong et al. [55] used SLB-
429 IL61 as second dimension in combination with 2-*O*-ethyl-3-*O*-ethyl-6-*O*-TBDMS-β-cyclodextrin ,
430 (EtEt-TBDMS -CD) to evaluate the enantiomeric composition of genuine Australian TTOs, thus
431 avoiding coelutions observed with a PEG stationary phase in the second dimension. The same
432 research group [89] adopted SLB-IL59 as first dimension for the quantitative determination of
433 sesquiterpenes and diterpenic acids in *Copaifera multijuga* Hayne oleoresin, to improve the
434 separation and to achieve a better occupation of the 2D space in GC×GC.

435 Last but not least, Yan et al. [90] introduced a three-dimensional gas chromatography platform
436 combined with accurate mass spectrometry (3D GC–accToFMS), in which an IL-based column (SLB-
437 IL59) was selected as 3rd dimension to analyse the agarwood (*Aquilaria malaccensis* Lam.)
438 oleoresin and the hop (*Humulus lupulus* L.) EO. This study demonstrated the need for a third SP for
439 these complex samples, since the classical apolar/PEG column set-up was not sufficient to provide
440 the separation of all the components.

441

442 **7 Instrument miniaturization**

443 The miniaturization of the instrumentations has recognisable benefits in terms of saving energy
444 and materials, and laboratory space and, moreover, it offers the possibility of in-field applications,
445 preserving natural resources and living organisms. Specifically, in the EOs field miniaturized GC
446 systems (μ GC) can be used to monitor the in-field volatile emission of the plants (e.g. helping to
447 detect the balsamic time) and in the on-line production of EOs or extracts.

448 Two approaches can be adopted to obtain μ GCs: i) the scaling-down all their components (i.e.
449 injector, column, detector) or ii) the development of micro-systems for GC by adopting the
450 microelectromechanical-system technology (MEMS) [91, 92].

451 Most studies in the literature concerning μ GC based on MEMS are focused on MEMS-based
452 columns [91-94], probably because separation step is the most important aspect during a GC run.
453 In this respect, in 2016, Cagliero et al. [95] developed highly efficient MEMS-based columns (N/m
454 above 8000) for the analysis of the plant volatiles, in particular for EOs. This study evaluated the
455 performance of a set of planar columns characterized by different dimensions and coated with
456 apolar (5%-phenyl-polymethylsiloxane), polar (auto-bondable nitroterephthalic acid-modified
457 polyethylene glycol) and chiral (EtEt-TBDMS- β -CD) SPs, by comparing the results with those
458 obtained with conventional NB columns. Their performance was evaluated in terms of column
459 inertness, efficiency and plate number per meter by analysing different EO samples, i.e. bergamot,
460 chamomile, lavender, lemon, peppermint, rosemary and sage. This study clearly demonstrated
461 that the EO profiles obtained with planar columns coated with all the above selected stationary
462 phase is perfectly overlapping to that of conventional NB GC column. Moreover, the authors
463 demonstrated also the high reliability of linear retention indices for all markers in sage EO. Similar
464 results were obtained on the quantitation performance of the MEMS-based columns, both in
465 terms of percentage abundance and absolute amount if compared to those of conventional GC
466 columns. Figure 7 reports the GC profiles of peppermint EO obtained with (A) FFAP-EXT planar

467 column, (B) Sil-5%-PH planar column, (C) the reference Sil-5%-PH NB column [with permission
468 from 95]. This example highlighted that the stationary phase selectivity played a crucial role in
469 obtaining the necessary retention and separation; the results with Sil-5%-PH planar column were
470 perfectly comparable, if not better, than those obtained with the conventional NB column. These
471 results also show that the MEMS-based columns can be successfully adopted for EO analyses and
472 open new perspective in the field.

473

474 **8 Concluding remarks**

475 Conventional GC-FID/MS analysis is a well-established approach to characterize the chemical
476 composition of an EO, and to assess its quality, authenticity and genuineness. However, in the last
477 decades, several improvements in the strategy of analysis of these matrices have been developed
478 showing the need of a continuous research in the field, in particular for samples of a medium-to
479 high complexity such as EOs. Their accurate characterization requires the constant development of
480 advanced analytical separation techniques (such as multidimensional GC) and/or the development
481 of new stationary phases with the required selectivity (e.g. cyclodextrin derivatives or ionic
482 liquids).

483 A further important aspect is the ever increasing number of analytical controls required in EO
484 field, that make mandatory the adoption of fast GC techniques to increase the sample throughput
485 and laboratory productivity and at the same time enable the total analysis costs. The literature
486 survey has evidenced that, although several Fast-GC methods have been developed for EO
487 analysis, they are not commonly adopted for new studies. In this respect, new perspectives could
488 arise from the development of miniaturized columns and instruments not only able to run Fast-GC
489 but also to minimize energy consumption and laboratory space.

490

491 **Conflict of interest statement**

492 The authors declare no conflict of interest.

493

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497

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761

762

Caption to the Figures

763 **Figure 1.** Peppermint EOs TIC patterns resulting from a) conventional GC-qMS analysis, b) F-
764 GCqMS at 20° C/min and c) F-GC-qMS at 60°C/min. Legend: 1) α -pinene, 2) β -pinene, 3) sabinene,
765 4) limonene, 5) 1,8-cineole, 6) γ -terpinene, 7) 3-octanol, 8) linalool, 9) menthofuran, 10)
766 isopulegol, 11) menthone, 12) neomenthol, 13) terpinen-4-ol, 14) isomenthone, 15) menthol, 16)
767 istd, 17) pulegone, 18) menthyl acetate, 19) trans- β -caryophyllene, 20) germacrene D [with
768 permission from 19].

769

770 **Figure 2.** F-GC analysis of lime EO using a 5 m, 50 μ m d_c x 0.05 d_f μ m capillary column and fast
771 temperature programming. Legend: 1) α -Thujene, 2) α -Pinene, 3) Camphene, 4) Sabinene, 5) β -
772 Pinene, 6) Myrcene, 7) Octanal+ α -Phellandrene, 8) α -Terpinene, 9) p-Cymene+Limonene, 10) E- β -
773 Ocimene, 11) γ -Terpinene, 12) cis-Sabinene hydrate, 13) Terpinolene, 14) Linalool, 15) Terpinen-4-
774 ol, 16) α -Terpineol, 17) Decanal, 18) Nerol, 19) Neral, 20) Geraniol+Piperitone, 21) Geranial, 22)
775 Neryl acetate, 23) Geranyl acetate, 24) α -Elemene, 25) cis- β -Bergamotene, 26) α -Caryophyllene,
776 27) trans- α -Bergamotene, 28) α -Humulene+E- β -Farnesene, 29) Santelene, 30) α -Selinene, 31) Z- α -
777 Bisabolene, 32) (E-E)- α -Farnesene+ β -Bisabolene, 33) Germacrene B, 34) 2,3-Dimethyl-3-(4-methyl-
778 3-pentenyl)-2-norbornanol, 35) Campherenol, 36) α -Bisabolol [with permission from 23].

779

780 **Figure 3.** Enantioselective GC-MS profiles of bergamot EO analysed with columns coated
781 respectively with MeMe-TBDMS CD (a), MeEt-TBDMS CD (b), EtMe-TBDMS CD (c), EtEt-TBDMS CD
782 (d), all dissolved in PS-086. Legend: 5) α -pinene, 6) β -pinene, 7) sabinene, 3) limonene, 57)
783 linalool, 20) linalyl acetate, 73) α -terpineol; a: (R) enantiomer, b: (S) enantiomer) [with permission
784 from 39].

785

786 **Figure 4.** EsGC × GC–FID contour plots of a TTO sample obtained using a ¹D MEGA-DEX DET-Beta
787 phase and (A) SUPELCOWAX®10 ²D column, (B) SLB-IL59 ²D column, (C) SLB-IL61 ²D column, and
788 (D) BPX5 2D column. Legend: 1(a), (–)-limonene; 1(b), (+)-limonene; 2(a), (+)-terpinen-4-ol; 2(b),
789 (–)-terpinen-4-ol; 3a, (–)-α-terpineol; 3(b), (+)-α-terpineol; 4, α-pinene; 5, α-terpinene; 6, 1,8-
790 cineole; 7, p-cymene; 8, γ-terpinene; X (unknown); and Y (unknown) [with permission from 55].

791

792 Figure 5. GC-FID patterns of sandalwood (*S. spicatum* (R. Br.) A. DC) EO with a) OV1, b) PEG-20M,
793 e) IL60, d) IL60i (optimized method) columns. Analysis conditions: (a-e) temperature program:
794 50°C_ (1min)//3°C/min//250°C_(2min), (d) temperature program: 70°C
795 (1min)//5°C/min//250°C_(2min), flow rate: 1 ml/min). Legend: 1) (Z,Z)-farnesol, 2) (E,Z)- farnesol,
796 3) (Z,E)-farnesol, 4) (E,E)-farnesol, 5) (Z)-a santalol, 6) (Z)-a-trans-bergamotol, 7)epi- santalol, 8)
797 (Z)- santalol, 9) bisabolol isomer, 10) nuciferol, ISTD: bacdanol [with permission from 85].

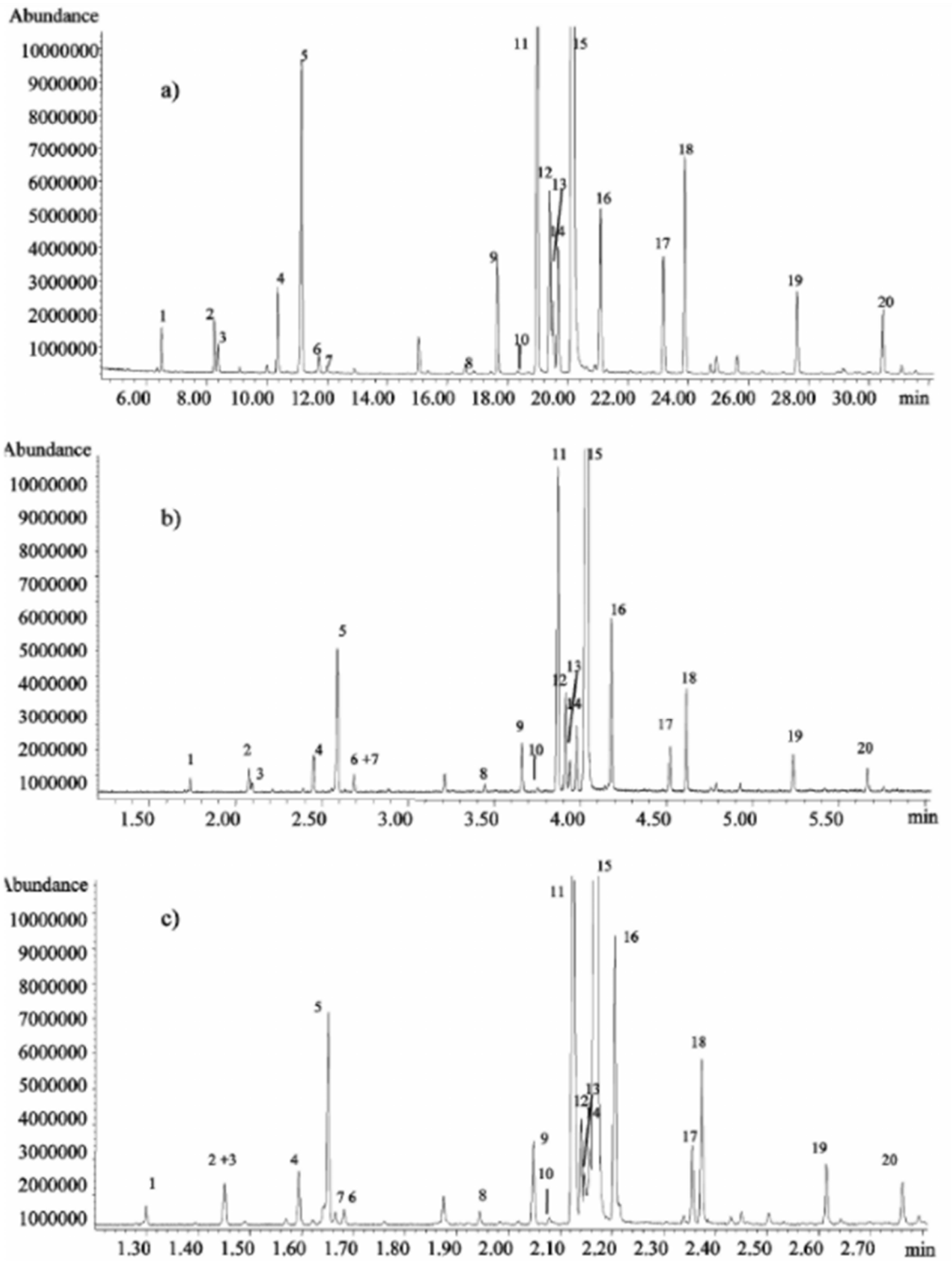
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799 Figure 6. GC-MS profiles of sage (*Salvia officinalis* L.) EO analyzed with [P₆₆₆₁₄⁺][Cl⁻] and
800 [P₆₆₆₁₄⁺][NTf₂⁻] 5 m test columns. Temperature program: from 50°C to 200°C (5 min) at 10°/min.
801 [with permission from 86].

802

803 Figure 7. GC profiles of peppermint EO obtained with (A) FFAP-EXT planar column, (B) Sil-5%-PH
804 planar column, (C) the reference Sil-5%-PH NB column. Analysis conditions: temperature program:
805 50°C//5°C/min//190°C for (A) and (C) and 495 50°C//2°C/min//190°C for (B). Legend: 1)
806 menthone, 2) isomenthone, 3) menthyl acetate, 4) neomenthol, 5) isomenthol, 6) menthol, 7)
807 neoisomenthol, 8) α-pinene, 9) β-pinene, 10 1,8-cineole, 11) limonene, 12) menthofuran, 13)
808 terpinen-4-ol, 14) β-caryophyllene, 15) germacrene D [with permission from 95].

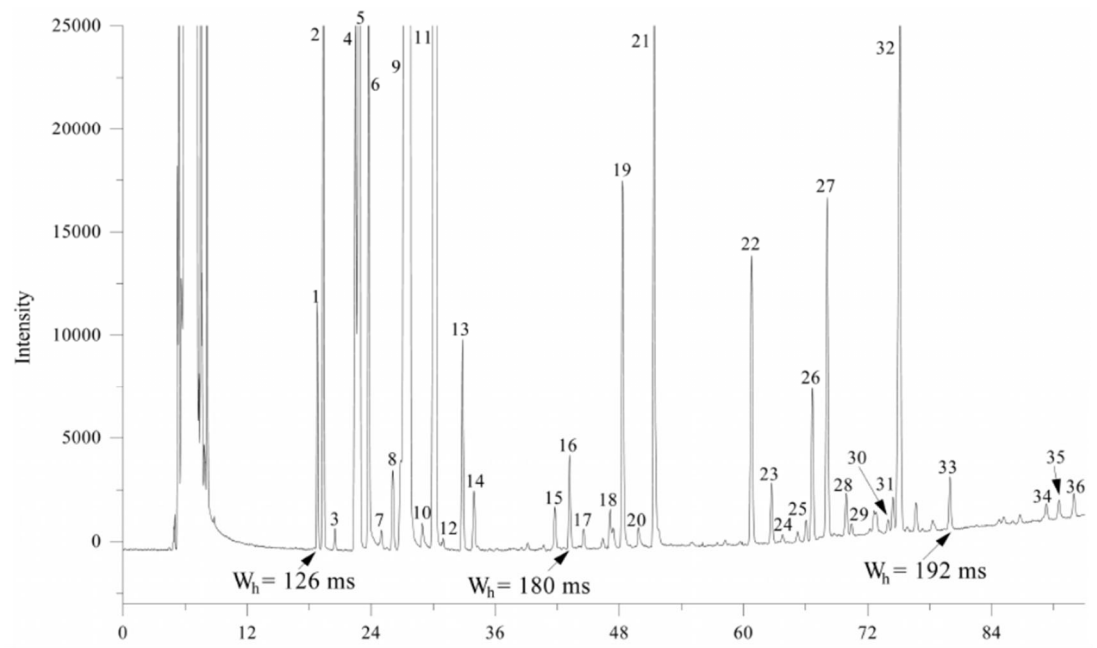
Figure 1



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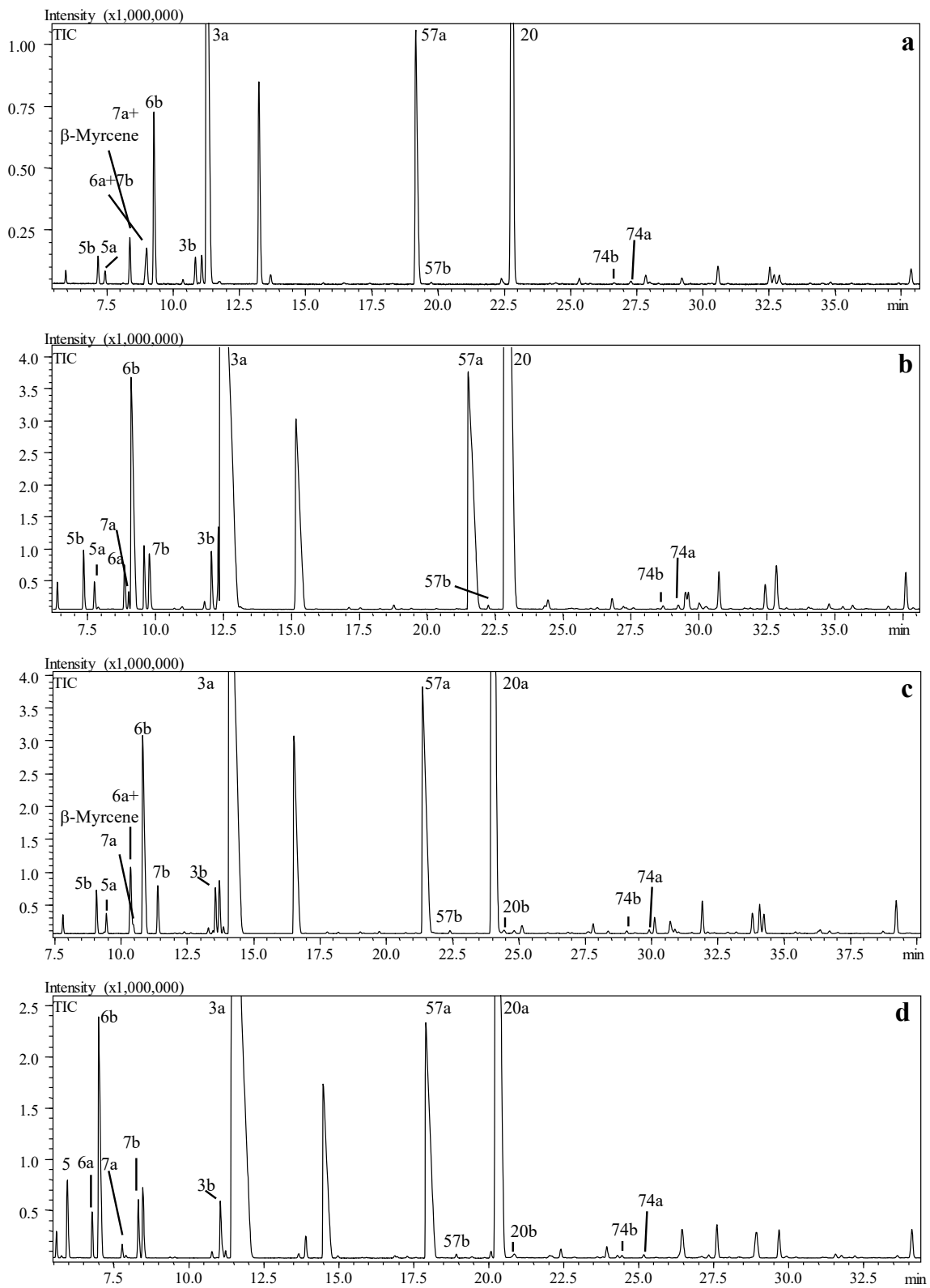
Figure 2



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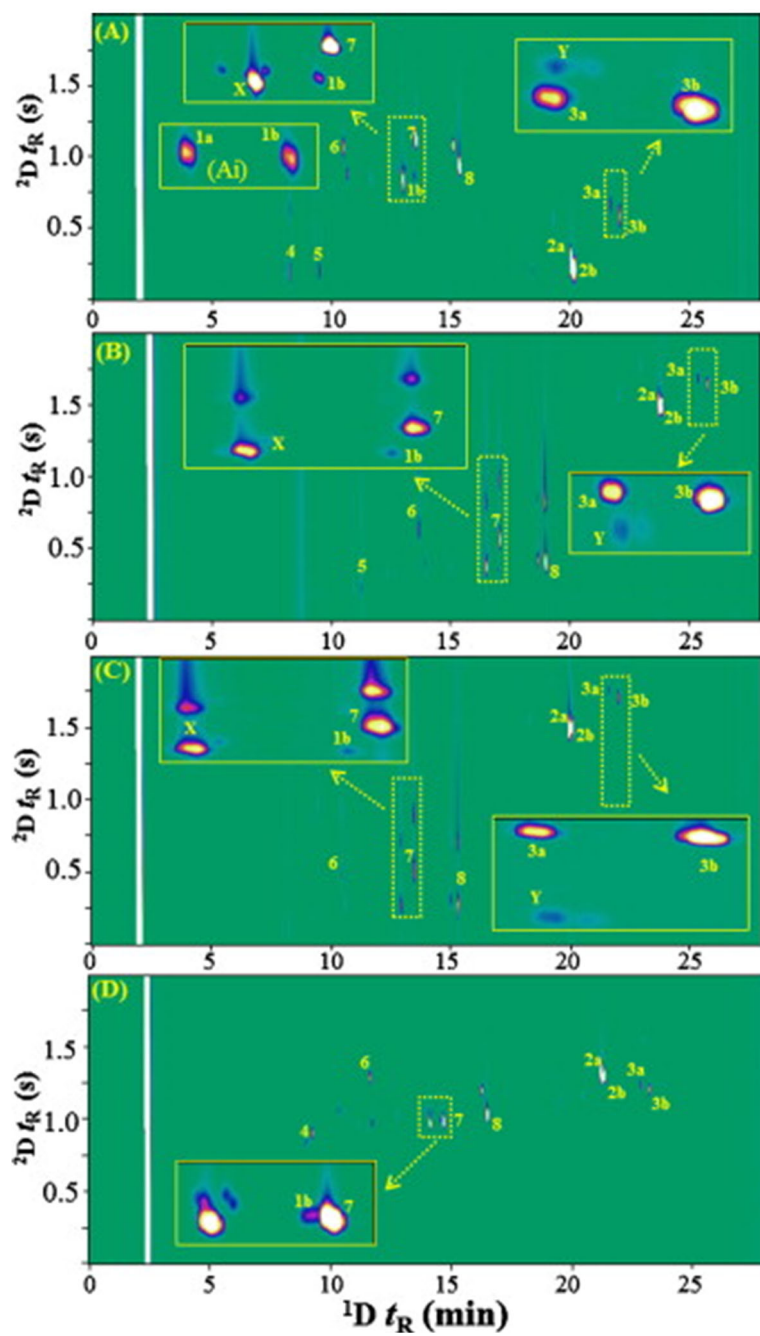
Figure 3



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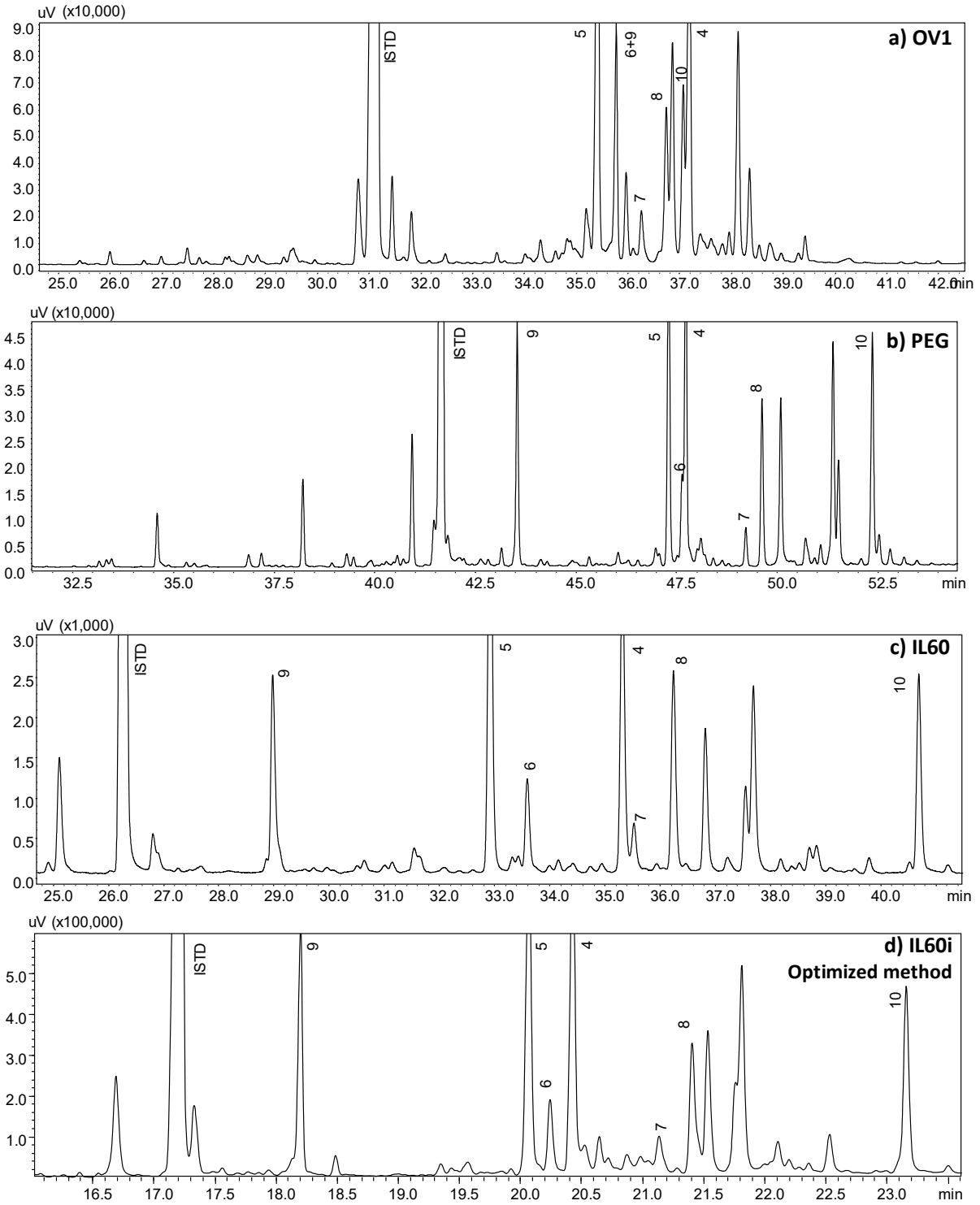
Figure 4



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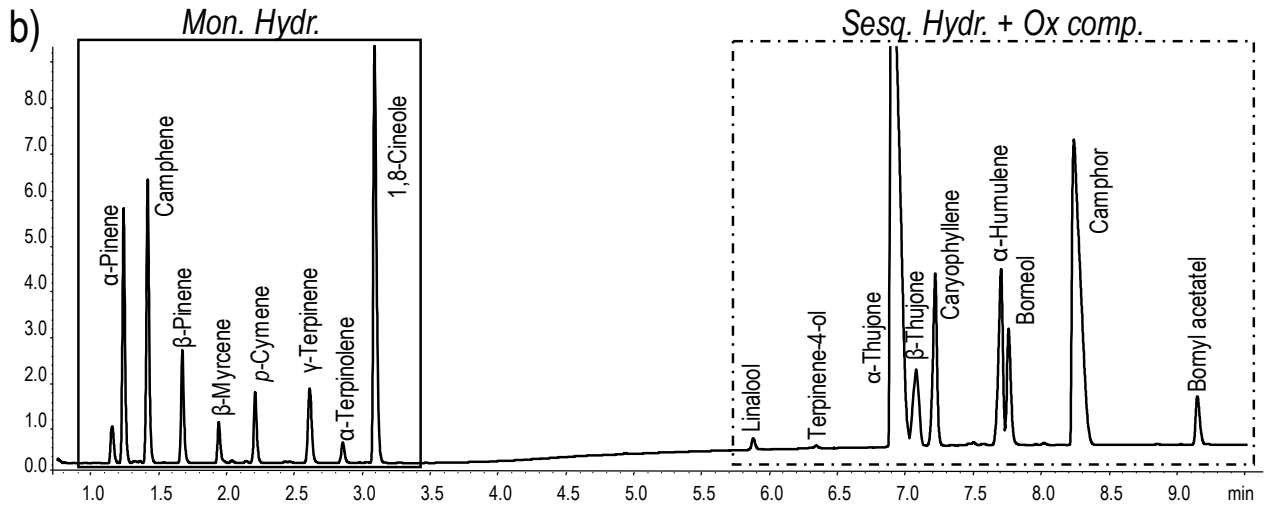
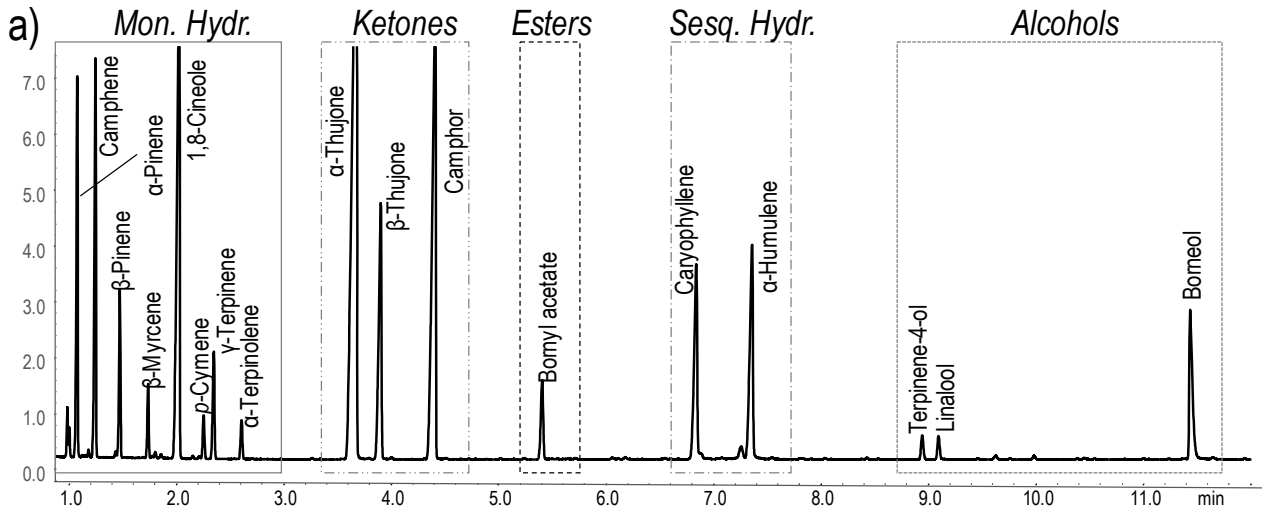
Figure 5



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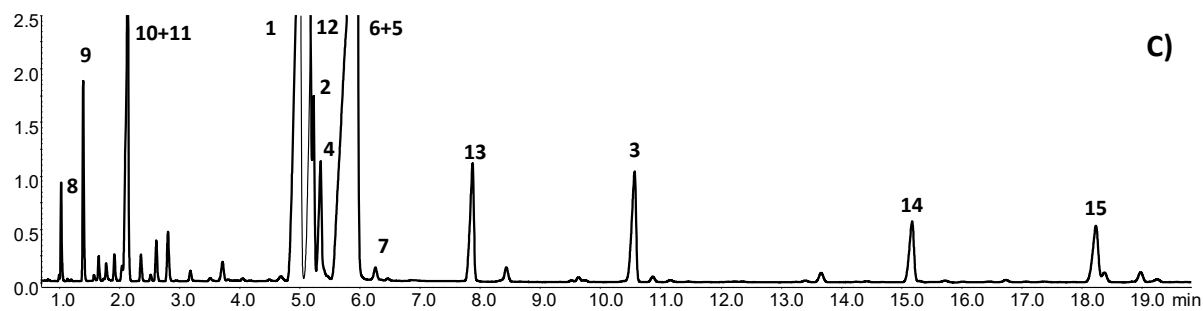
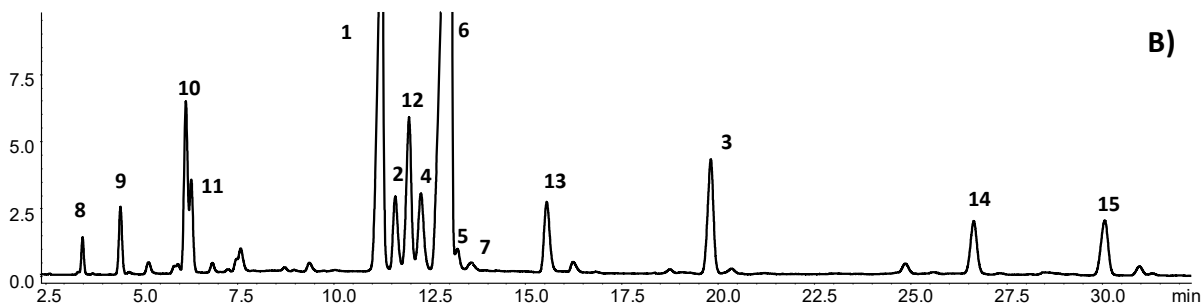
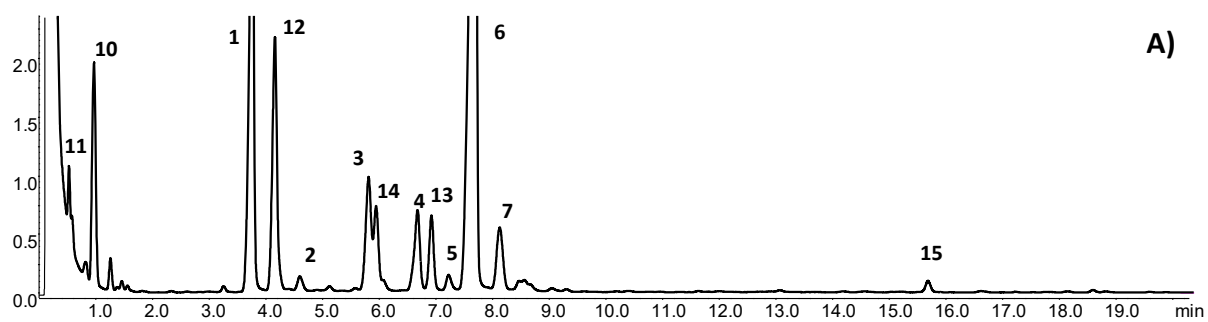
Figure 6



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Figure 7



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