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**Can the selectivity of phosphonium based ionic liquids be exploited as stationary phase for routine gas chromatography? A case study: The use of trihexyl(tetradecyl) phosphonium chloride in the flavor, fragrance and natural product fields**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1742468> since 2020-06-29T16:33:58Z

*Published version:*

DOI:10.1016/j.chroma.2020.460969

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(Article begins on next page)

1 **Can the selectivity of phosphonium based ionic liquids be exploited as stationary**  
2 **phase for routine gas chromatography? A case study: the use of trihexyl(tetradecyl)**  
3 **phosphonium chloride in the flavor, fragrance and natural product fields**

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18

19 **Abstract**

20 Room temperature ionic liquids (ILs) are well established stationary phases (SPs) for gas chromatography  
21 (GC) in several fields of applications because of their unique and tunable selectivity, low vapor pressure and  
22 volatility, high thermal stability (over 300°C), and good chromatographic properties. This study is focused on  
23 an IL based on a phosphonium derivative (trihexyl(tetradecyl)phosphonium chloride, [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>]), previously  
24 shown to be suitable as a gas chromatographic SP because of its unique selectivity. In particular, it aims to  
25 establish the operative conditions to apply [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] to routine analysis of samples containing medium to  
26 high volatility analytes with different polarity, organic functional groups and chemical structure. In the first  
27 part, the study critically evaluates long term [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] column stability and maximum allowable operating  
28 temperatures (MAOT). The relatively low MAOT (210°C) requires the adoption of a dedicated approach for  
29 analytes eluting above this temperature based on a suitable combination of efficiency and selectivity, and  
30 column characteristics (length, inner diameter and film thickness) and operative conditions. The performance  
31 of [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] as a GC SP have been validated through the Grob test, a model mixture of 41 compounds of  
32 different polarity, structure, and with different organic functional groups in the flavor and fragrance field, a  
33 standard mixture of 37 fatty acid methyl esters, some essential oils containing pairs or groups of compounds  
34 of different volatility critical to separate in particular peppermint, thyme, oregano, sandalwood and  
35 frankincense. The above approach has produced highly satisfactory separations with all of the samples  
36 investigated.

37

38 **Keywords:** gas chromatography; ionic liquid stationary phases; phosphonium based ionic liquids; selectivity,  
39 efficiency and operative temperatures; flavors, fragrances and natural products

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## 42 1. Introduction

43 Room temperature ionic liquids (ILs) are nowadays successfully applied in several fields because of their  
44 unique and tunable selectivity, low vapor pressure and volatility, high thermal stability (over 300°C), and  
45 good chromatographic properties [1, 2].

46 The peculiar characteristics of ILs have made them of great interest, also for the flavor, fragrance and  
47 essential oil (EO) fields where new stationary phases (SPs) with selectivities different from those of routinely-  
48 used polysiloxane and polyethylene glycol derivatives are continuously sought after [3]. This need is related  
49 to the complexity of the samples in these fields that are often mixtures of several isomeric and/or  
50 homologous components with similar structural and physical characteristics (e.g. mono- and  
51 sesquiterpenoids in EOs). Their separation is therefore mandatory for the correct identification and  
52 quantitation and implies the indispensable complementary contribution of diagnostic chromatographic data  
53 (e.g. retention indices) and their mass spectra [4-6].

54 In a previous article, the uncommon selectivity of two phosphonium based ILs, namely, (trihexyl(tetradecyl)  
55 phosphonium chloride,  $[P_{66614}^+][Cl^-]$ , and trihexyl(tetradecyl)phosphonium bis[(trifluoromethyl)  
56 sulfonyl]imide,  $[P_{66614}^+][NTf_2^-]$ , was described. These derivatives were chosen based on the results of a  
57 systematic study by Breitbach and Armstrong [7], who in 2008 determined the coefficients of the Abraham  
58 relationship on eleven phosphonium based ILs. They showed that the ability of the two above ILs to interact  
59 with solutes through their hydrogen bond basicity (the *a* coefficient in the relationship) significantly differed  
60 (6.60 for  $[P_{66614}^+][Cl^-]$  vs 1.55 for  $[P_{66614}^+][NTf_2^-]$ ) unlike the *e*, *s*, *b*, and *l* coefficient that were rather similar (*e*  
61 is indicative of interactions through  $\pi$  and nonbonding electrons, *s* of dipolarity, *b* of H-bond acidity, and *l* of  
62 dispersion forces).

63 Recently, this research group showed that their performance as GC SPs were highly complementary since a  
64  $[P_{66614}^+][Cl^-]$  test column provided strong selectivity based on analyte functional groups and a very high  
65 retention of oxygenated compounds. Meanwhile, the corresponding one coated with the  $[P_{66614}^+][NTf_2^-]$  IL  
66 separated analytes depending on their polarity and volatility [8]. The study concluded emphasizing the need  
67 for further experiments to validate these columns for their use in routine analysis of complex real-world  
68 samples.

69 The present study is an in-depth and wide breath investigation on how to exploit the peculiar and uncommon  
70 selectivity of  $[P_{66614}^+][Cl^-]$  and to establish the optimal operative conditions for routine analysis of medium to  
71 high volatility analytes with different polarity and chemical structure. It mainly deals with optimization of  
72 column maximum allowable operating temperature (MAOT) and long-term stability, and of column efficiency  
73 and geometry (including length, inner diameter and film thickness), and performance. The tests have here  
74 been carried out with a test mixture of 41 compounds of different polarity, structure and with different  
75 functional groups in the flavor and fragrance field (FFMIX), a standard mixture of 37 fatty acid methyl esters  
76 (FAMES), some essential oils containing pairs or groups of compounds critical to separate.

77

## 78 2. Experimental

79

### 80 2.1 Samples and chemicals

81 Trihexyl(tetradecyl)phosphonium chloride [ $P_{66614}^+$ ][Cl<sup>-</sup>] (~97%) was purchased from Merck (Milan, Italy). The  
82 IL was used without further purification.

83 The following mixture or samples were used for this study:

84 i) the Grob test [9], (**1**: decane, **2**: dodecane, **3**: 1-octanol, **4**: 2,3-butanediol, **5**: methyl decanoate, **6**: methyl  
85 undecanoate, **7**: methyl dodecanoate, **8**: 2,6-dimethylphenol, **9**: 2,6-dimethylaniline, **10**: dicyclohexylamine,  
86 and **11**: 2-ethylhexanoic acid) in hexane and trichloromethane, was purchased from Merck (Milan, Italy) and  
87 analyzed as received.

88 ii) a flavor and fragrance standard mixture (FFMix) consisting of 41 compounds:  $\beta$ -pinene (**1**), limonene (**2**),  
89 nonane (**3**)(ISTD), undecane (**4**)(ISTD), tridecane (**5**)(ISTD), 1,8-cineole (**6**), camphor (**7**), menthone (**8**), *i*-  
90 menthone (**9**), pulegone (**10**), linalyl acetate (**11**), bornyl acetate (**12**), menthyl acetate (**13**), lavandulyl  
91 acetate (**14**), terpinyl acetate (**15**), ethyl 2-methylbutanoate (**16**), *trans*- $\beta$ -caryophyllene (**17**), estragole (**18**),  
92 anethole (**19**),  $\gamma$ -hexalactone (**20**),  $\gamma$ -heptalactone (**21**),  $\gamma$ -octalactone (**22**), neral (**23**), geranial (**24**), carvone  
93 (**25**), 2-methylbutanol (**26**), 1-octanol (**27**), terpinen-4-ol (**28**), linalool (**29**),  $\alpha$ -terpineol (**30**), *neo*-menthol  
94 (**31**), *neo-i*-menthol (**32**), menthol (**33**), *i*-menthol (**34**), lavandulol (**35**), borneol (**36**), viridiflorol (**37**), eugenol  
95 (**38**), *i*-eugenol (**39**), carvacrol (**40**), thymol (**41**). All compounds were from Merck (Milan, Italy) or from  
96 author's standard collection. They were solubilized at a concentration of 100 mg L<sup>-1</sup> each in cyclohexane.

97 iii) a Fatty Acid Methyl esters (FAMES) standard solution from Merck consisting of 37 compounds dissolved  
98 in methylene chloride (C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1c, C15:0, C15:1c, C16:0,  
99 C16:1c, C17:0, C17:1c, C18:0, C18:1n9c, C18:1n9t, C18:2n6c, C18:2n6t, C18:3n6c, C18:3n3c, C20:0, C20:1n9c,  
100 C20:2n6c, C20:3n3c, C20:3n6c, C20:4n6c, C20:5n3c, C21:0, C22:0, C22:1n9c, C22:2n6c, C22:6n3c, C23:0,  
101 C24:0, and C24:1n9)

102 iii) essential oils (EO) of peppermint (*Mentha x piperita* L.), thyme (*Thymus vulgaris* L.) and oregano  
103 (*Origanum vulgare* L.) were obtained by hydrodistillation following the procedure of the European  
104 Pharmacopoeia [10]. The santalols, farnesols and bergamotol were kindly provided by Dr. D. Joulain, Robertet  
105 (Grasse, France) as well as the essential oils of *Santalum album* L. and *S. yasi* Seem.; they were solubilized in  
106 cyclohexane at a concentration of 5 g L<sup>-1</sup> before analysis. The frankincense sample of *Boswellia socotrana*  
107 Balf.f. EO, and pure standards of incensol, serratol and incensyl acetate were all provided by Prof. G.  
108 Appendino (Università del Piemonte Orientale, Novara, Italy)

109 All solvents were all HPLC grade from Merck (Milan, Italy).

110

### 111 2.2 Analysis conditions

112 2.2.1. Instrumental set-up

113 Analyses were carried out on a Shimadzu GC-FID 2010 unit equipped with Shimadzu GC Solution 2.53U  
114 software and a Shimadzu GC 2010 – Shimadzu QP2010-PLUS GC-MS system equipped with GCMS 2.51  
115 software (Shimadzu, Milan, Italy). FID was used to measure chromatographic parameters, while MS was used  
116 for identification purposes.

117 2.2.2. Columns

118 The list of the [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] columns investigated together with their characteristics and performance are  
119 reported in Table 1. Columns with different characteristics were prepared by Mega (Legnano (MI), Italy) using  
120 the static coating procedure after a proprietary deactivation process of the fused silica surface.

121 2.2.3. GC-MS and GC-FID conditions

122 GC-MS analyses were carried out under the following conditions: temperatures: injector: 240°C; transfer line:  
123 240°C, ion source: 200°C; carrier gas: He, flow control mode: constant linear velocity, flow rates: for  
124 conventional (i.e. 0.25 mm)  $d_c$  columns: 1 mL/min, for 0.18 mm  $d_c$  column: 0.7mL/min, for narrow bore (0.10  
125 mm  $d_c$ ) columns: 0.4 mL/min. Each column was connected to the MS through a post-column of deactivated  
126 fused silica (0.5 m x 0.10, 0.18 or 0.25 mm  $d_c$ ) (Mega, Legnano, Italy) to make it compatible with the interface  
127 temperature higher than MAOT. Injection conditions were: mode: split; split ratio: 1:20 for columns *I, II, VI,*  
128 *VII* and 1:50 for columns *III, IV, V*, volume: Grob test: 2  $\mu$ L, all other samples 1  $\mu$ L. Oven temperature programs  
129 are reported in the captions of the corresponding figures.

130 GC-FID analyses were carried out under the following conditions: temperatures: injector: 240°C; detector:  
131 240°C; carrier gas: H<sub>2</sub>. Flow rates: 1 mL/min for conventional  $d_c$  columns, 0.7 mL/min for 0.18 mm  $d_c$  column,  
132 0.4 mL/min for 0.10 mm  $d_c$  columns. All other analysis conditions were the same as those reported in the  
133 previous GC-MS paragraph. FID sampling rate: 40 ms. Oven temperature programs are reported in the  
134 captions of the corresponding figures.

135 *Analyte identification:* when necessary, analytes were identified through their mass spectra and/or linear  
136 retention indices. Mass spectra were compared to those of authentic standards or to those of commercial or  
137 in-house libraries, or literature data. Linear retention indices of the available standards were calculated  
138 *versus* a 100 mg L<sup>-1</sup> C9-C25 hydrocarbon solution, home-made with pure standards provided by Merck (Milan,  
139 Italy), analyzed under the conditions reported above.

140 2.2.4. Column characterization

141 Each column was characterized through the following parameters (Table 1):

142 i) separation measure,  $\Delta s$ , calculated on FFMIX between camphor (**7**) and borneol (**36**) analyzed under the  
143 conditions reported above and in the corresponding figures [11]

144 ii) the number of total theoretical plates (N) and the number of theoretical plates per meter (N/m) for each  
145 column were measured through the analysis of naphthalene under isothermal GC conditions giving retention  
146 factors (k) between 10 and 30.

147

### 148 3. RESULTS AND DISCUSSION

149 3.1 Characterization of  $[P_{66614}^+][Cl^-]$  stationary phase and evaluations of the performance of columns of  
150 different geometry

151 The first part of this study concerned the stability of the columns coated with  $[P_{66614}^+][Cl^-]$ , the determination  
152 of operative conditions and limits, the consistency of their performance over time, and the MAOT.

153 The first experiments (with a 25 m, 0.25mm  $d_c$ , 0.25  $\mu m$   $d_f$  column) provided evidence of a film instability  
154 resulting in the formation of droplets after conditioning, which directly affects the column efficiency. This  
155 instability was also noted for other columns with film thickness above 0.25  $\mu m$  for 0.25  $d_c$  columns. This  
156 shortcoming was overcome by reducing the film thickness by about 30% and thus increasing the phase ratios  
157 for columns with the same inner diameter. The resulting reduction of retention times, in particular, for the  
158 highly retained analytes with free hydroxyl group(s) in their structure, has the positive effect to shorten the  
159 total analysis time. On the other hand, hydrocarbons and low polarity compounds are poorly retained,  
160 although always well separated (see below). The stability of the thinner film column was conventionally  
161 studied with two  $[P_{66614}^+][Cl^-]$  test columns *I* (Table 1); one of the columns was conditioned at increasing  
162 temperatures with isothermal steps (i.e., 200, 220, 240 and 270°C) of 12 hours and its performance evaluated  
163 with the Grob test and the baseline behavior. This column showed good inertness up to 270°C but, at the  
164 same time, it presented two important drawbacks. The first drawback was a notable increase of the baseline  
165 starting from 210°C that did not decrease even after repeated and prolonged conditioning cycles; the second  
166 one, apparently not related to the previous one, was a significant and continuous loss of retention strictly  
167 connected with the progress of the conditioning process at high temperature. The loss of retention was  
168 evaluated through the retention time of the 2,6-DMP peak (2,6-dimethylphenol) i.e., one of the last eluting  
169 peaks in the Grob test carried out at the end of each conditioning cycle. Figure 1a reports the GC pattern of  
170 the Grob tests and of 2,6-DMP (**8**) under the usual conditions applied for this analysis after conditioning the  
171 column *I* for 12 hours at 220°C and at 270°C. The loss of 2,6-DMP retention is 2.6 min (156 sec).

172 Two hypotheses were raised to explain the concurrent unstable bleeding at high temperature and the loss  
173 of retention (i.e., SP loss because of decomposition or evaporation).

174 The decomposition of  $[P_{66614}^+][Cl^-]$  due to the applied temperature was excluded because Armstrong and  
175 Breitbach [7] showed that its thermal stability limit is 335°C. This value was confirmed in the authors'  
176 laboratory by differential scanning calorimetry (DSC) analysis (data not reported). A further indirect  
177 confirmation of thermal stability was the GC-MS analysis at the operative temperatures where no signal  
178 related to its decomposition diagnostic ions (i.e.,  $m/z$  483, 398 and 286 were recorded with GC-MS in SIM  
179 mode up to 240°C (data not reported) [12].

180 The second hypothesis explored the possible loss of stationary phase due to its evaporation at high  
181 temperature. This possibility has been substantiated in a 2018 study of Deferm et al. that studied in depth

182 the thermal stability of [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] [12]. Starting from the consideration that, like any other liquid, ILs have  
183 a vapor pressure (although extremely low), they showed that they can evaporate when left in permanent  
184 contact with an inert gas flowing over or through them. This results in a mass loss without IL decomposition.  
185 Very few data on the IL vapor pressure are available and, to the best of the authors' knowledge, those related  
186 to [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] are not known. These authors studied the behavior of purified [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] with static  
187 thermogravimetric analysis (TGA) analysis at ambient pressure and under nitrogen atmosphere and found a  
188 mass loss over 24 hours of 0.8% at 165°C, 1.3% at 180°C, about 3% at 220°C, about 35% at 270°C and  
189 decomposition at 320°C. The same TGA experiments over 12 hours were repeated in the authors' laboratory  
190 with a commercial 97% pure [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] each 10°C within the range 180-220°C to measure its MAOT as SP  
191 in GC. The results were comparable to those of Deferm et al [12] with similar percentage mass losses, i.e. 1.0%  
192 at 180°C, 1.2% at 190°C, 1.3% at 200°C, 1.4% at 210°C and 3.4% at 220°C.

193 After these experiments, the mass loss after one hour was separately measured, because this time has been  
194 assumed as the maximum duration for the final isothermal step in a programmed temperature GC analysis  
195 of real-world samples. In this case, the mass loss was 0.02% at 180°C, 0.02% at 190°C, 0.03% at 200°C, 0.05%  
196 at 210°C and 0.2% at 220°C.

197 These results indicated that the MAOT affording good stability for columns coated with [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] can be  
198 fixed at 210°C. This value is also in agreement with the practical rule identified for IL GC stationary phases to  
199 consider as MAOT a temperature around 100°C lower than their decomposition temperature [13, 14]. A cycle  
200 of 20 runs of the Grob Test from 40°C to 210°C with 10 min of final isothermal step was carried out on the  
201 second column *I* after the usual conditioning at MAOT and resulted in a perfect overlapping of the 2,6-DMP  
202 retention times and a very stable baseline. Figure 1b reports the GC patterns of the Grob test after the  
203 injection n°1, n°10 and n° 20 and the enlargement of the 2,6-DMP (**8**) peak in these injections.

204 These conditions enable the unique selectivity of this IL SP to be exploited for those samples whose analytes  
205 of interest elute below 210°C. For example, this is the case of several essential oils, as shown in the previous  
206 article [8]. Figure 2 reports the GC pattern of peppermint (*Mentha x piperita* L.) essential oil analyzed with  
207 column *I* and *III*, whose components are clearly separated as a function of their organic functional groups,  
208 the last one of them (viridiflorol, (**26**)) eluting at 190°C with column *I* and at 185°C with column *III*.

209 But how about the possibility of exploiting the unique [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] selectivity and high retention with analytes  
210 eluting above 210°C with conventional columns (e.g. column *I*)? The trend of the last two decades in GC  
211 separation has constantly been to increase the separation power of GC columns by improving efficiency by  
212 both refining their technology of preparation and/or acting on their dimensions, i.e., as it has effectively and  
213 well been summarized by Blumberg and Klee, by "... *killing the separation with plates and dimensions* ..." [15].  
214 The solution for less volatile analytes and/or those retained above the MAOT of [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] columns is to  
215 reduce their elution temperature. A possibility to obtain this goal is to reduce the column length while  
216 keeping the same inner diameter and film thickness, of course, with a concomitant loss of efficiency (i.e.



217 number of total theoretical plates N). However, it is well known that for a large number of applications, the  
218 efficiency of the routine capillary columns is often much higher than necessary, and a reduction should not  
219 affect the success of a high number of separations. In addition, when the column efficiency decreases, the  
220 selectivity of the stationary phase can significantly contribute to achieve the required separation [16].

221 In this part of the study, the concurrent peculiar selectivity and high retention for oxygenated compounds of  
222 [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] has been exploited to lead these analytes to elute below column MAOT by acting on their  
223 dimensions.

224 A set of columns with different characteristics (length, inner diameter and film thickness) coated with the  
225 [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL as GC SP were tested with standard mixtures and real world sampling by fixing 210°C as column  
226 MAOT. Table 1 reports the list of columns tested together with their characteristics. The experiments have  
227 been carried out on i) a standard mixture of 41 compounds with different structure, volatility and polarity in  
228 the flavor and fragrance field (FFMIX), ii) essential oils with highly retained components (thyme and oregano  
229 EOs), iii) a standard mixture of 37 fatty acid methyl esters (FAMES), iv) a mixture of sesquiterpene alcohols  
230 characteristic of sandalwood EO (santalols, farnesols and bergamotol) and two original EOs, and iv) a  
231 standard mixture of diterpenoids characteristics of frankincenses (*Boswellia* spp.) (incensol, incensyl acetate  
232 and serratol) and the *Boswellia socotrana* Balf.f. EO. Since the main aim of this study was to evaluate how to  
233 exploit the selectivity of the [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL at temperatures compatible with its MAOT, all analyses were  
234 carried out under the same GC conditions, (i.e., without optimizing separations for each samples and/or  
235 minimizing analysis time for each by determining dedicated temperature programs and flow rates with  
236 efficiency optimized flow (EOF) and without translation of the methods) [17]. The applied temperature rates  
237 for all columns were 2, 5 and 10°C/min.)

238 Each column was characterized in terms of efficiency and separation power on the FFMIX by determining the  
239 number of theoretical plates (N/m and N) calculated on naphthalene under GC conditions giving retention  
240 factors (k) between 10 and 30 and separation number,  $\Delta s$ , calculated between camphor (**7**) and borneol (**36**).  
241 (Table 1) [11]. Figure 3 reports the GC patterns of FFMIX obtained with five of the investigated columns.  
242 Figure S1 highlights the part of the GC-FID patterns including the oxygenated analytes eluting between  
243 camphor (**7**) and borneol (**36**) on the five columns.

244 A column with conventional characteristics (column *I*; l: 30 m;  $d_c$ : 0.25 mm;  $d_f$ : 0.15  $\mu$ m) was used to evaluate  
245 the performance of [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] as GC SP with a MAOT of 210°C. This column showed an efficiency of 2700  
246 theoretical plates per meter (N/m) for a total of 81000 theoretical plates (N) calculated on naphthalene at  
247 100°C with a retention factor  $k=10$ . The FFMIX was analysed with a temperature program of 5°C/min up to  
248 210°C: 39 compounds on 41 were eluted and all separated at the base line with the exceptions of the critical  
249 pairs limonene (**2**)/1,8-cineole (**6**) and bornyl acetate (**12**)/lavandulyl acetate (**14**), and anethole (**19**)/ $\gamma$ -  
250 hexalactone (**21**) that partially overlapped. Thymol (**40**) and carvacrol (**41**) were not eluted under the adopted  
251 conditions. The analyte separation was in agreement with the polarity of their organic nature; for example,

252 hydrocarbons, carbonyl and hydroxyl containing compounds, with the exception of *trans*- $\beta$ -caryophyllene  
253 (**17**), a C15 sesquiterpene hydrocarbon, with a lower volatility. Shorter columns (column **VI**, 10m, and column  
254 **VII**, 5m) with the same  $d_c$  and  $d_f$  were also tested. With these columns, all 41 compounds eluted including  
255 thymol (**40**) and carvacrol (**41**) although they were not separated. However, in spite of the [P<sub>66614</sub><sup>+</sup>] [Cl<sup>-</sup>] high  
256 selectivity, the efficiency of the 10 m column (N: 27,000) was not sufficient to enable the separation of the  
257 pairs pulegone (**10**)/anethole (**18**) and  $\alpha$ -terpineol (**30**)/*neo*-menthol (**31**) in addition to limonene (**2**)/1,8-  
258 cineole (**6**). The results with the 5 m column worsened also in the non-aromatic hydroxylated compound  
259 region, although without coelutions. In any case, the good selectivity within the groups of the carbonyl  
260 derivatives and of the non-aromatic hydroxylated compounds was maintained. (Figure S1). Column **II** (20 m  
261 x 0.18 mm x 0.12  $\mu$ m) showed similar performance with the same coelutions and the non-elution of thymol  
262 (**40**) and carvacrol (**41**). Its main advantage is that the analysis time is reduced from about 40 minutes to 33  
263 minutes compared to column **I**. Its efficiency calculated by naphthalene at 90°C ( $k=11$ ) increased at 4500 N/m  
264 for a total N of 90000.

265 Column **III** (10m x 0.10mm x 0.06  $\mu$ m) had an efficiency calculated by naphthalene at 85°C ( $k=22.5$ ) of 6100  
266 N/m for a total N of 61000. This column afforded the elution of thymol (**40**) and carvacrol (**41**) although they  
267 were not baseline separated at about 210°C. A temperature rate not higher than 5°C/min must be applied to  
268 elute thymol (**40**) and carvacrol (**41**) because higher rates induce an increase of their elution temperature  
269 above the MAOT of 210°C. As expected, column **III** kept the separation of the critical pairs very similar to that  
270 of the previous columns producing the baseline separation of anethole (**21**) and heptalactone (**19**), but not  
271 of the pairs limonene (**2**)/1,8-cineole (**6**), pulegone (**10**)/estragole (**18**), and  $\alpha$ -terpineol (**30**)/*neo*-menthol  
272 (**31**)

273 The selectivity of the [P<sub>66614</sub><sup>+</sup>] [Cl<sup>-</sup>] IL SP compensated for the reduction of the efficiency of column **IV** due to  
274 the shortening to 5 m ( N= 30500) keeping the separation similar to that of column **III** with the exception of  
275 the pulegone (**10**)/menthyl acetate (**13**) and neral (**23**)/carvone (**25**) pairs, while limonene (**2**)/1,8-cineole  
276 (**6**),and  $\alpha$ -terpineol (**30**)/*neo*-menthol (**31**) pairs were not separated as with column **III**. On the other hand,  
277 column **IV** provided the separation, although partial, of thymol (**40**)/carvacrol (**41**) at a low temperature rate  
278 (2°C/min).

279 A further shortening of the length to 3 m (Column **V**) implied a further reduction of efficiency (N = 18300)  
280 but the separation at a temperature rate of 2°C/min overlaps with that obtained with column **IV** under the  
281 same conditions. This column provided a base-line separation of the thymol (**40**)/carvacrol (**41**) pair,  
282 indicating that, under these conditions, selectivity again well compensates for the loss of efficiency.

283 These results highlight the observation of the evolution of the separation number  $\Delta s$  (Table 1) within the set  
284 of columns investigated.  $\Delta s$  was here calculated between camphor (**7**) and borneol (**36**) because i) this is the  
285 most complex part of the chromatograms and ii) thymol (**40**) and carvacrol (**41**) do not elute with columns **I**  
286 and **II**.  $\Delta s$  varies, as expected, reaching a maximum value (928) with column **III** and the minimum with column

287 **VII** (507) revealing that in spite of the drastic reduction of efficiency, selectivity still strongly drives the  
288 separation. This is even more evident with the results of the narrow bore columns (**III**, **IV**, and **V**) where in  
289 spite of a drastic reduction of  $\Delta s$  (from 928 to 679) and N (61000 vs. 18300) the separation of the carbonyl-  
290 containing analytes between column **V** (3 m) column **III** (10 m) is comparable.

### 291 3.2 Applications to real-world samples

292 Some important EOs such as those of thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.) contain  
293 thymol (**40**) and carvacrol (**41**) as main and/or characterizing components and can be derived from different  
294 plant chemotypes. Their elution and separation is therefore fundamental to determine their quantity or  
295 relative abundance to establish their origin, quality or possible adulterations. The above results on FFMIX  
296 showed that only columns **III**, **IV** and **V** can be used for the analysis of these EOs, although column **III** has to  
297 be avoided because under the applied analysis condition it does not separate thymol (**40**) from carvacrol (**41**).  
298 Figure 4 reports the GC patterns of thyme and oregano EOs analyzed on column **IV** where it is evident that  
299 this column has enough selectivity and efficiency to provide the separation of all characterizing compounds  
300 including monoterpene hydrocarbons.

301 The second mixture used to evaluate the [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL as GC SP with a MAOT at 210°C is a commercial  
302 standard mixture of 37 FAMES from C4 to C24. FAMES are one of the most popular and successful fields of  
303 applications of IL as GC stationary phases and their use has recently extensively been reviewed by Fanali et  
304 al. in 2017 [18] while their separation with commercially-available IL columns has been assessed in 2014 by  
305 Dettmer [10]. This IL SP immediately showed very high selectivity for this class of compounds since it very  
306 well separates FAMES as a function of their number of carbon atoms and provides baseline separation of  
307 nearly all the saturated and unsaturated analogues within the same number of carbon atoms (e.g., six  
308 components on seven in the C18 cluster and six on eight in the C20 cluster). However, a MAOT of 210°C in  
309 combination with high retention made possible the elution of all FAMES up to C24 only with columns **IV** and  
310 **V**; with column **I** the elution was limited to C18 cluster, with column **II** to C20 and with column **III** at C22.  
311 Figure 5 reports the GC patterns of the 37 FAME standard mixture analyzed with columns **IV** and **V**. Figure S3  
312 enlarges the part of the GC-FID pattern where the C18-C24 FAME clusters elutes with column **IV**. The use of  
313 short conventional columns **VI** and **VII** (i.e. 10 and 5 m, 0.25mm  $d_c$ ), afforded the elution up to C20 and C24  
314 respectively, but their selectivity was not sufficient to compensate for the drastic reduction of efficiency (N=  
315 27000 and N= 13500, respectively) that resulted in the coelution of some C18 and C20 unsaturated analogues  
316 within their cluster.

317 The next group of experiments concerns the separation of compounds with medium-to-low volatility that  
318 represent important natural products, in particular, the sesquiterpenic alcohols of sandalwood EO and the  
319 diterpenoid markers of origin of frankincense. In both cases, the presence of hydroxyl groups in their  
320 chemical structure makes them retain above the MAOT of columns **I**, **II** and **III**.

321 Sandalwood EO is characterized by two groups of sesquiterpenic alcohols (C15), in particular four farnesol  
322 and four santalol isomers and bergamotol. Their presence and ratios are indicative of geographical and  
323 botanical origin, quality and possible adulterations or frauds [19]. The [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL SP enables the baseline  
324 separation of all compounds in a single run with both columns **IV** and **V**. Figure 6 reports the GC patterns of  
325 both two samples of *Santalum album* L. and *S. yasi* Seem. EOs and the standard mixtures of farnesols,  
326 santalols and bergamotol. The very high selectivity of [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] enables not only the baseline separation  
327 of the nine isomers even with a 3 m column, but also discriminates between acyclic (farnesols) and cyclic  
328 (santalols and bergamotol) isomers. Similar results have been obtained with shorter lengths of columns **I** (10  
329 and 5 m) showing that, in this case too, separation is driven by selectivity.

330 Frankincenses are the resins secreted by some species belonging to the genus *Boswellia* mainly originating  
331 from African Horn and Arabian Peninsula. Incensol and serratol (two C20 diterpenic alcohols) and incensyl  
332 acetate are used to distinguish their botanical and geographical origin. Incensol and serratol are not  
333 separated with apolar conventional columns, but are baseline separated with conventional PEG columns [20].  
334 In this case, columns **IV** and **V** provided a very good separation of the three markers with a very high  
335 resolution of 18.1 of the incensol/serratol pair with column **IV** and of 11.5 with column **V**. Columns **I**, **II** and  
336 **III** did not afford the elution of the two markers because they retained above their MAOT. Figures 7 a and b  
337 report the GC patterns on column **IV** of a sample of *B. sacotrana* Balf.f. EO and of the standards of incensyl  
338 acetate (**1**), serratol (**2**), and incensol (**3**).

339

#### 340 **4. Conclusions**

341 The [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL has been shown to be a successful IL stationary phase with unique selectivity based on  
342 organic functional groups and characterized by a retention that drastically increases with the analyte polarity,  
343 in particular for hydroxylated compounds. These characteristics makes it highly convenient for routine  
344 analysis of complex samples in the fields of flavors, fragrances and natural products. Its main limit is a rather  
345 low MAOT (i.e., 210°C) due to a relatively non-negligible vapor pressure. This limit is not only irrelevant for  
346 those analyses that can be completed below this temperature, but it can also be overcome for those that can  
347 be finalized below this temperature through a suitable combination of i) efficiency and selectivity, and ii)  
348 column characteristics and operative conditions. This approach was successfully applied to elute and  
349 separate analytes with medium to low volatility including the separation of FAME analogues within each  
350 cluster up to C24 and diterpenoidic alcohols up to C20. Further investigations are required for analytes that  
351 elute at temperatures above 210°C, but the require SPs with the same selectivity. Their analysis can be  
352 finalized either by introducing new phosphonium-based ILs with similar selectivity but lower vapour pressure,  
353 or by crosslinking the [P<sub>66614</sub><sup>+</sup>][Cl<sup>-</sup>] IL with external reagents or by appropriately modifying its structure to  
354 make it suitable to be immobilized to the fused silica wall. Investigations in this respect are under way.

355 The present study is part of a wide project aiming to evaluate new stationary phases for GC with unique  
356 selectivity as a support to the conventional and highly consolidated SPs in the flavor (aroma), fragrance and  
357 natural product fields.

358

#### 359 **Acknowledgements**

360 The authors are indebted to Robertet SA (Grasse, France) for the financial support to the Laboratory of  
361 Pharmaceutical Biology and Food Chemistry of the Dipartimento di Scienza e Tecnologia del Farmaco,  
362 Università di Torino. The authors are grateful to Prof. Dr. Roberta Cavalli (Dept. of Scienza e Tecnologia del  
363 Farmaco - University of Torino) for thermal analyses. J.L.A. acknowledges funding from the Chemical  
364 Measurement and Imaging Program at the National Science Foundation (Grant No. CHE 1709372).

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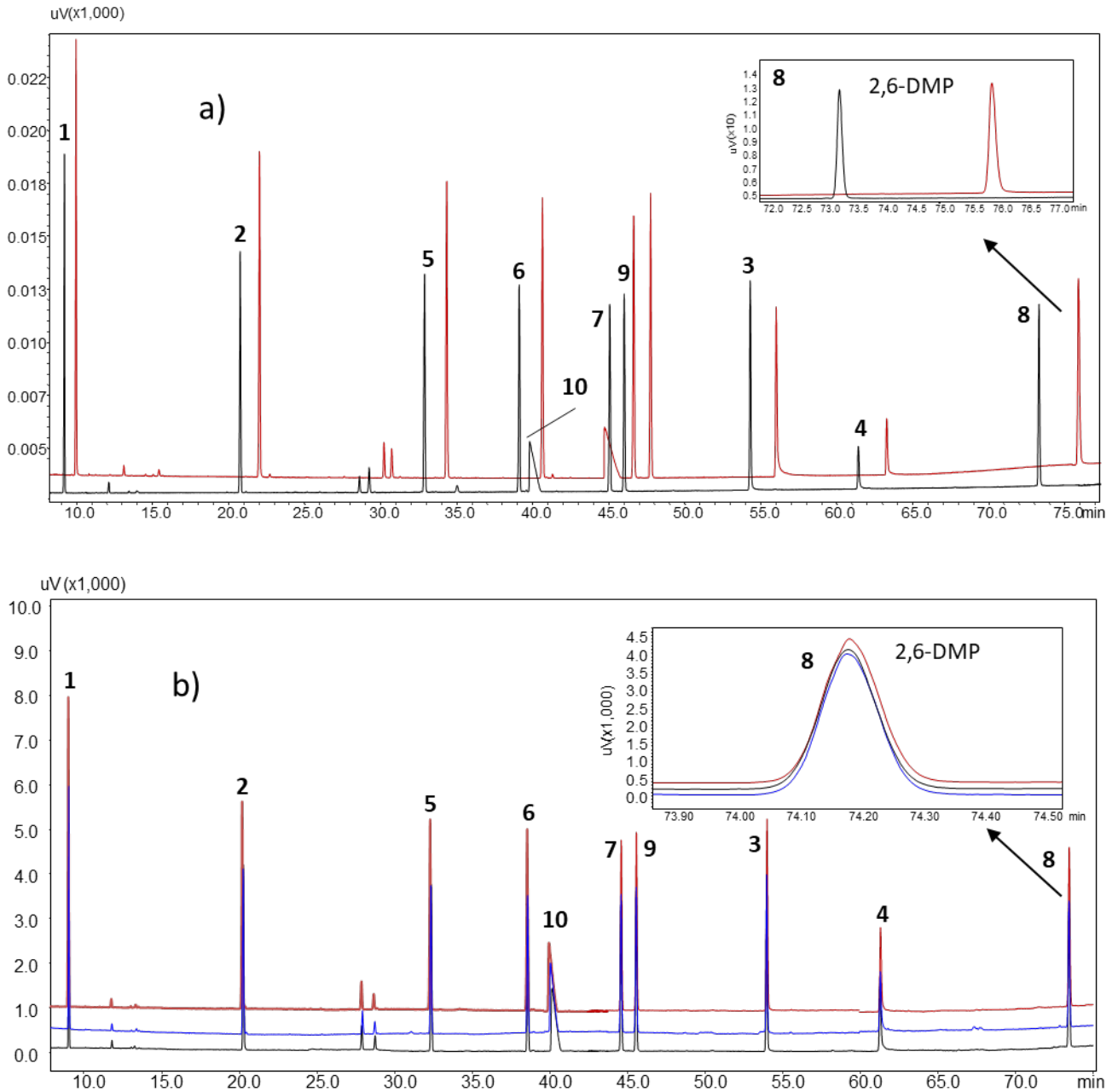
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414 extract dilution analysis and two-dimensional gas chromatography-mass spectrometry/olfactometry,  
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416 **Captions to figures**

417 Figure 1: GC-FID patterns of Grob test analyzed on column *I*: (a) patterns after 12 hours conditioning at 220°C  
418 (red) and 270°C (black); (b) patterns after 12 hours conditioning at 210°C: injection 1 (black), injection 10  
419 (blue), injection 20 (red). Temperature program: 50°C (1 min)//2°C/min//210°C (5 min). 2,6-DMP: 2,6-  
420 dimethylphenol. For the other analytical conditions, see section 2.2.3



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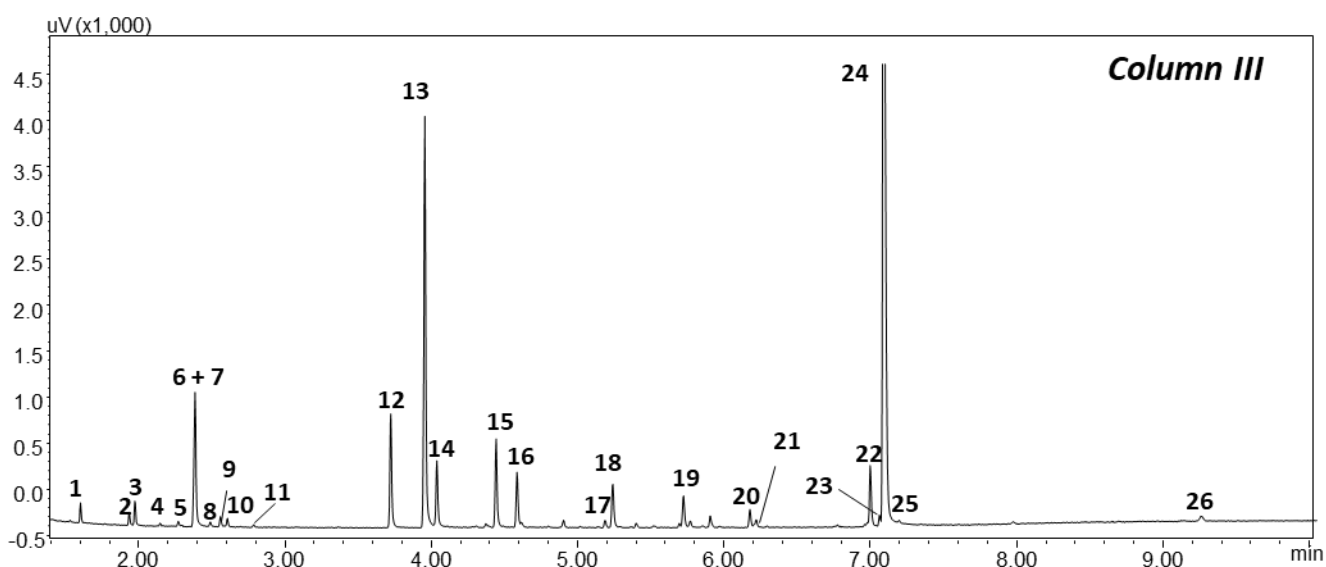
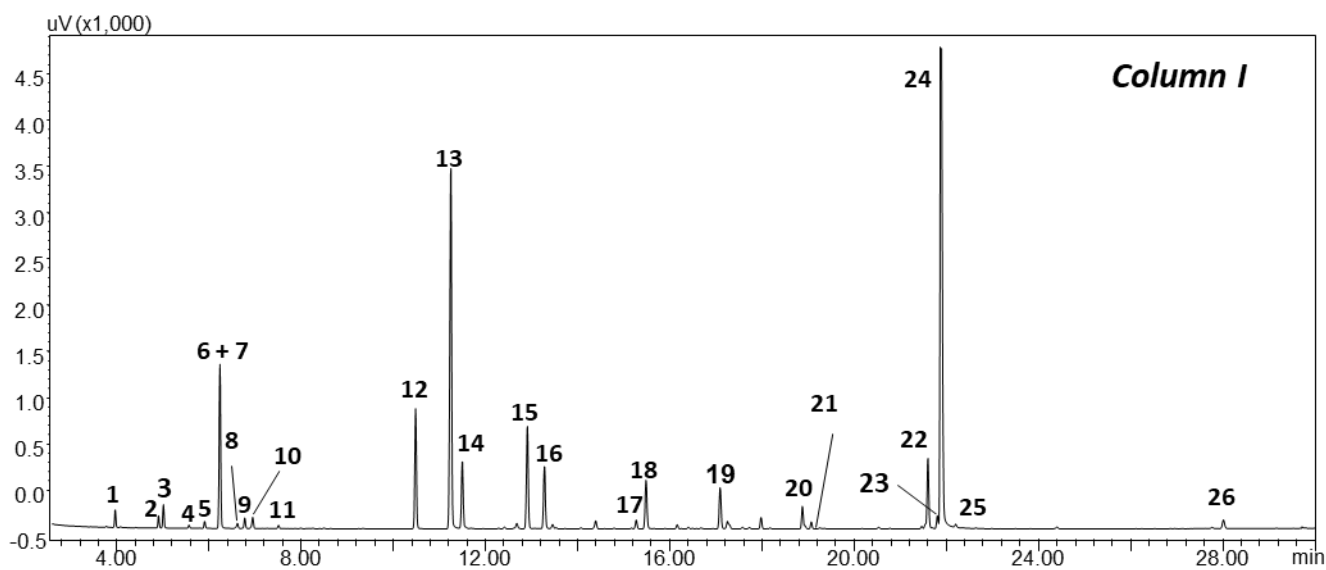
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424 Figure 2: GC-FID patterns of peppermint essential oil analyzed on column *I* (a) and *III* (b). Analysis conditions:  
425 temp. progr. Column *I*: 50°C (1 min)//5°C/min//210°C (5 min); column *III*: 50°C (1 min)//15°C/min//210°C (5  
426 min). For the other analytical conditions, see section 2.2.3.

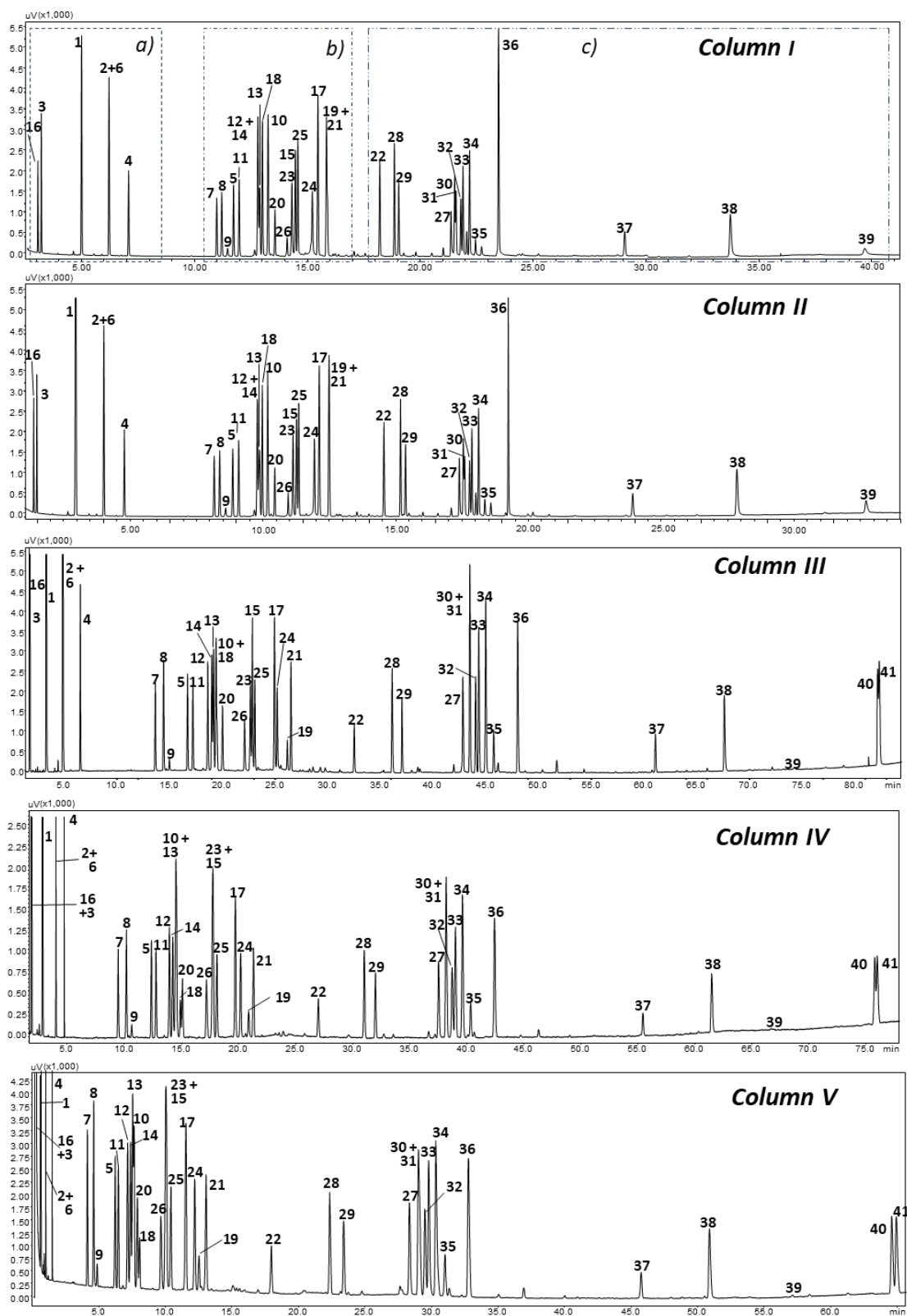
427 Peak identification: (1)  $\alpha$ -pinene, (2) sabinene, (3)  $\beta$ -pinene, (4)  $\beta$ -myrcene, (5)  $\alpha$ -terpinene, (6) 1,8-cineole,  
428 (7) limonene, (8) *cis*-ocimene, (9) *p*-cimene, (10)  $\gamma$ -terpinene, (11)  $\alpha$ -terpinolene, (12) menthofuran, (13)  
429 menthone, (14) *i*-menthone, (15) menthyl acetate, (16) pulegone, (17) piperitone, (18) caryophyllene, (19)  
430 germacrene-D, (20) 4-terpineol, (21) linalool, (22) neomenthol, (23) neo-*i*-menthol, (24) menthol, (25) *i*-  
431 menthol, (26) viridiflorol.



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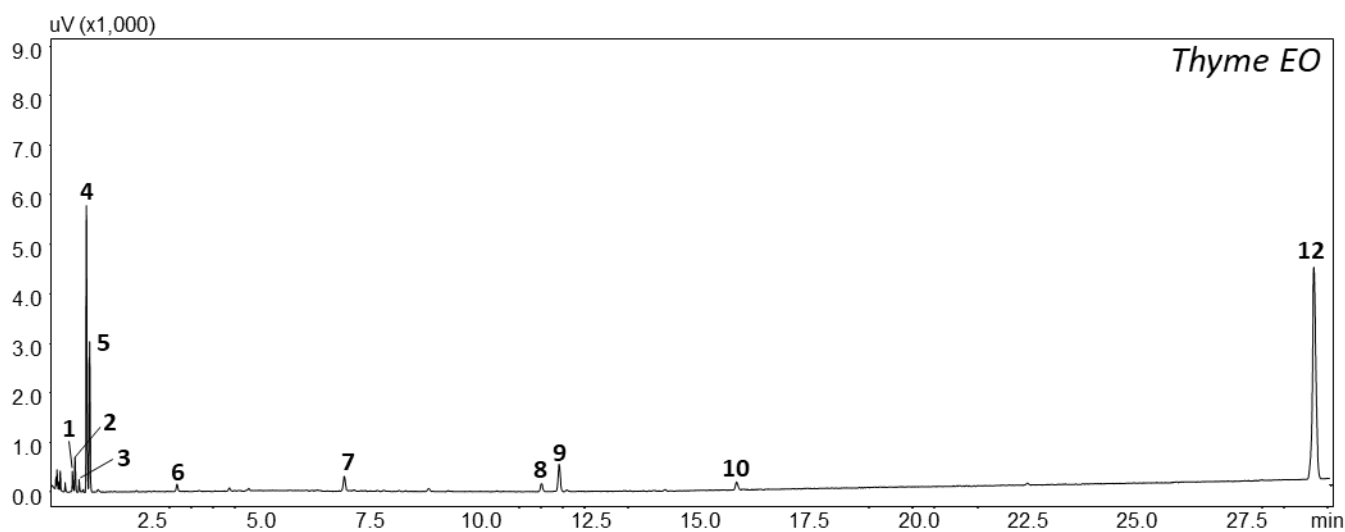
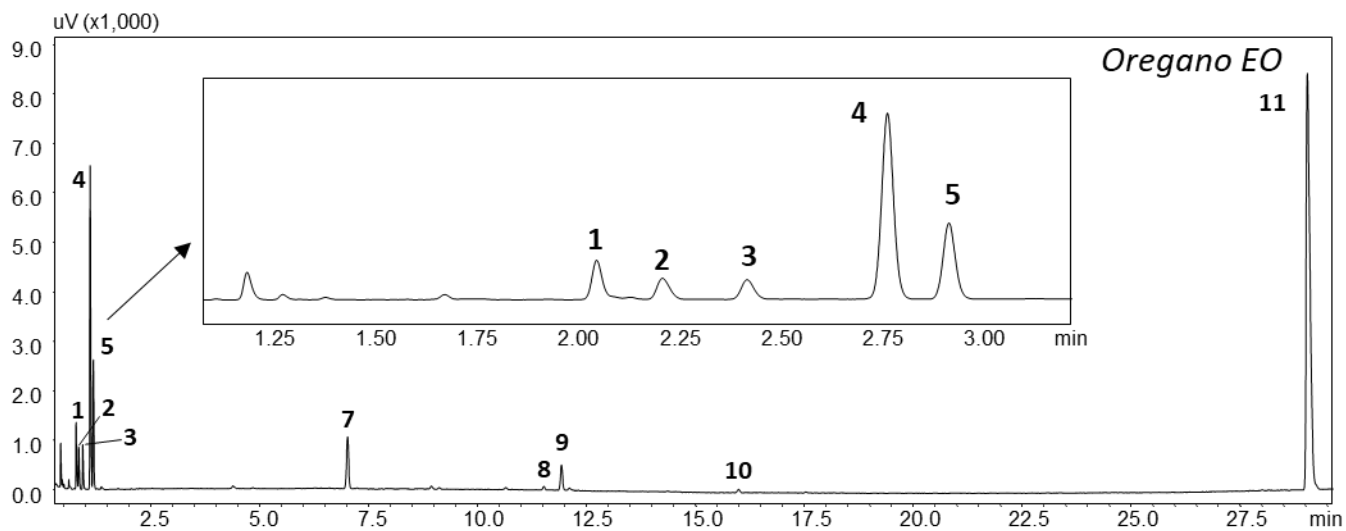
434 Figure 3: GC-FID patterns of FFMIX analyzed on the five  $[P_{66614}^+][Cl^-]$  columns investigated (Table 1). a)  
 435 hydrocarbons, b) carbonyl derivatives, c) hydroxyl derivatives. Analysis conditions: temp. progr. Columns *I*  
 436 and *II*: 50°C (1min)//5°C/min/2/10°C (5 min); columns *III*, *IV* and *5*: 50°C (1min)//2°C/min//210 (5 min). For  
 437 the other analytical conditions see section 2.2.3 and for peak identification paragraph 2.1.



438

439 Figure 4: GC-FID patterns of oregano and thyme essential oil analyzed on column **IV**. Analysis conditions:  
440 temp. progr.: 50°C (1min)//5°C/min//210°C (5 min). For the other analytical conditions see section 2.2.3

441 Peak identification:  $\beta$ -myrcene (**1**),  $\alpha$ -terpinene (**2**),  $\alpha$ -pinene (**3**), *p*-cimene (**4**),  $\gamma$ -terpinene (**5**), camphor (**6**),  
442 *trans*- $\beta$ -caryophyllene (**7**), terpinen-4-ol (**8**), linalool (**9**), borneol (**10**), carvacrol (**11**), thymol (**12**)



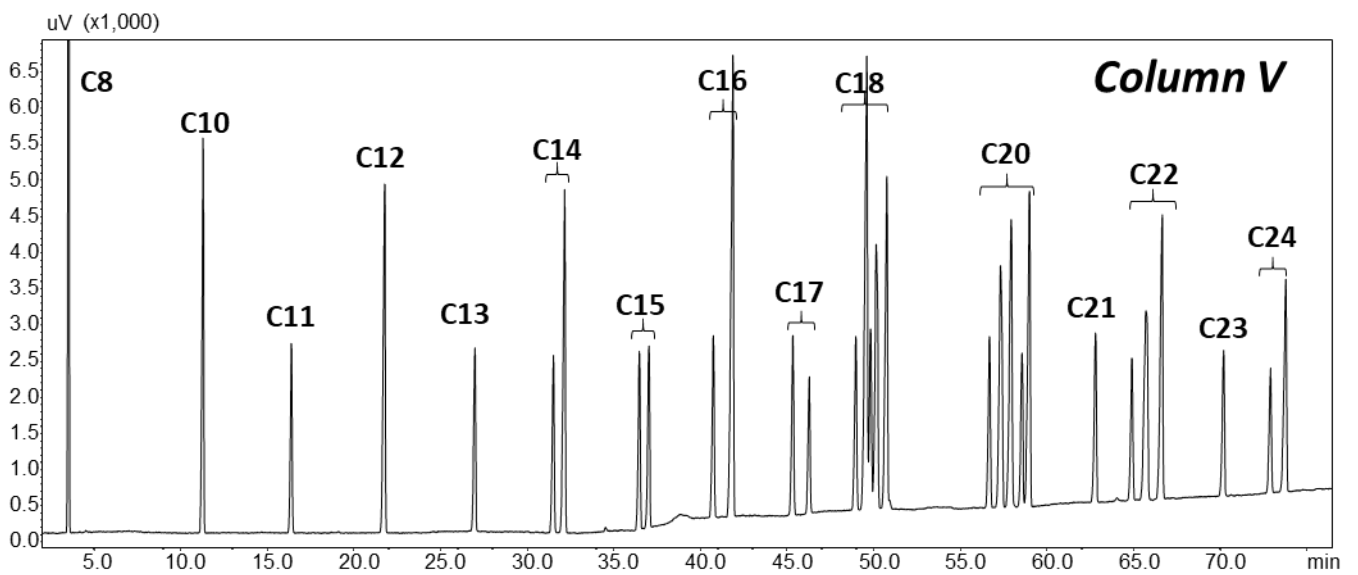
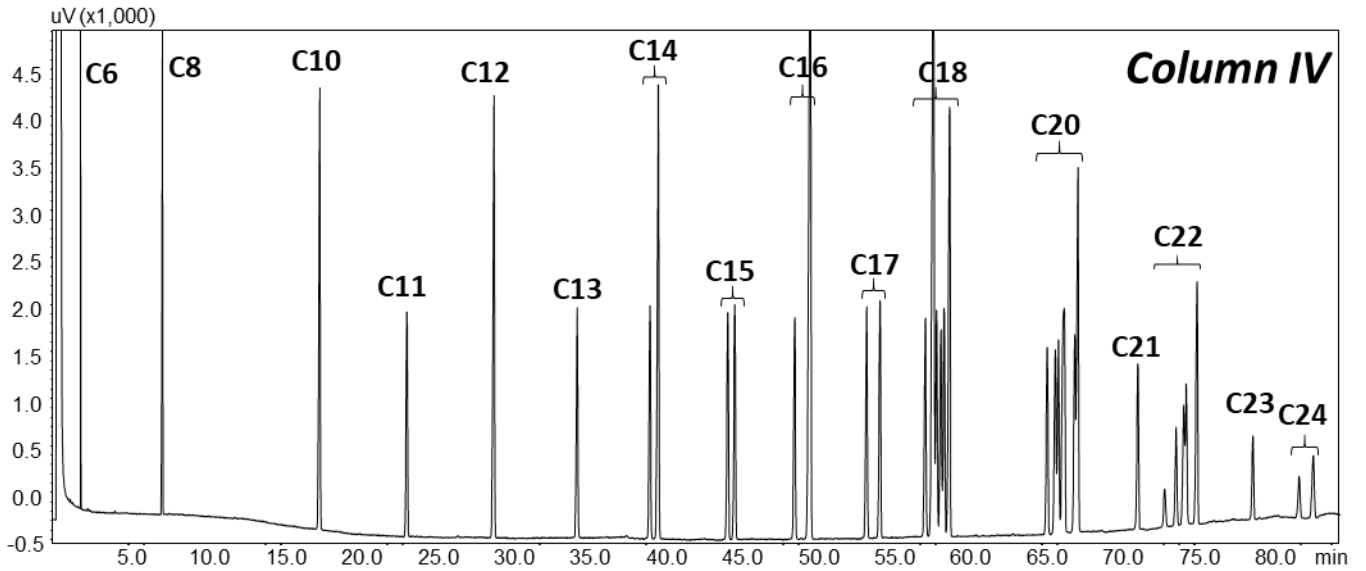
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445 Figure 5: GC-FID patterns of the 37 FAME standard mixture analyzed with columns *IV* and *V*.

446 Analysis conditions: temp. progr. Columns *IV* and *V*: 50°C (1min)//2°C/min//210°C (5 min). For the other

447 analytical conditions see section 2.2.3 and for cluster identification section 2.1.

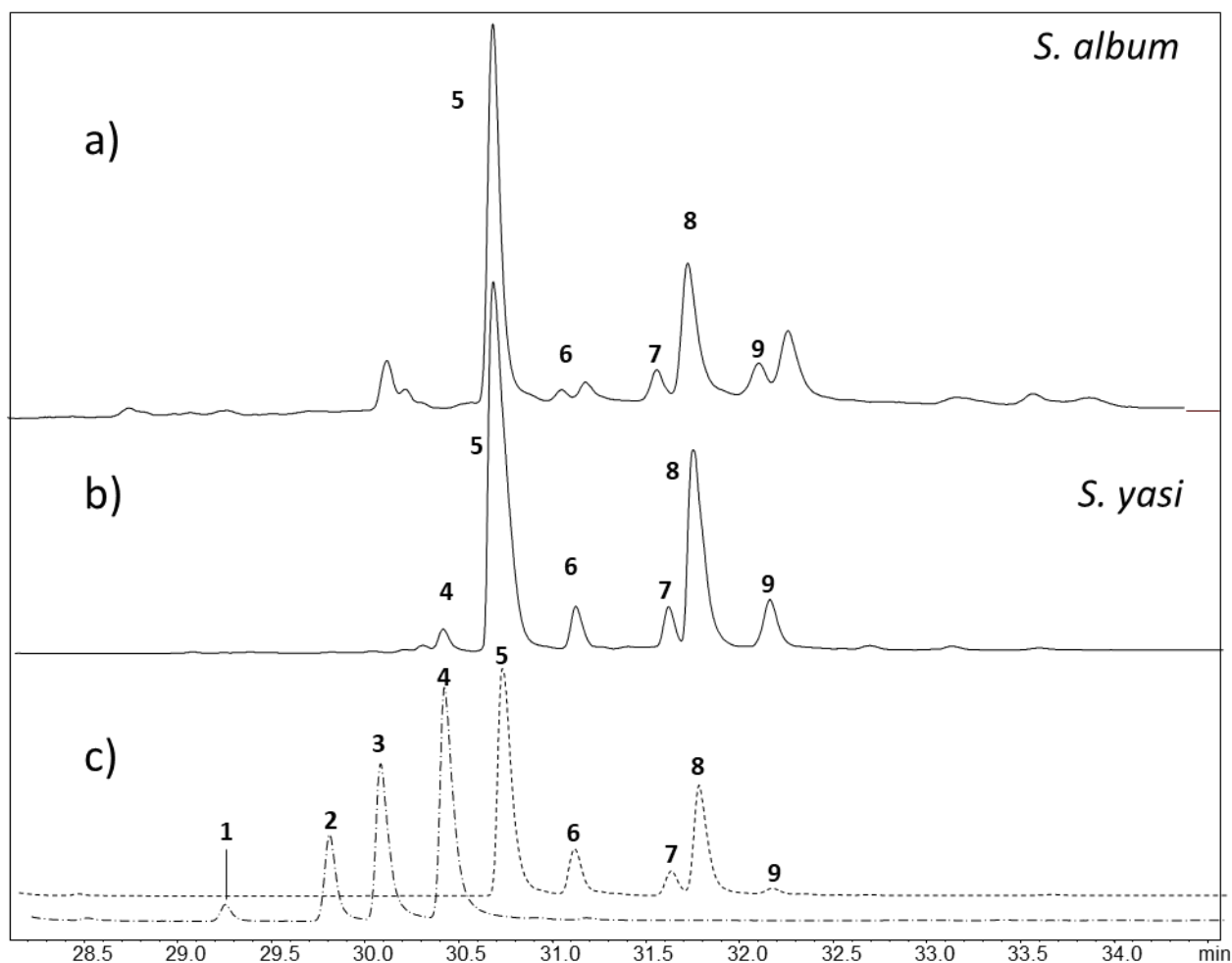


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450 Figure 6: GC-FID patterns with column **IV** of *S. album* (a) and *S. yasi* EOs (b) and of the standard mixture of  
451 farnesols, santalols and bergamotol (c). Analysis conditions: temp. progr.: 50°C (1 min)//5°C/min//210°C (5  
452 min). For the other analytical conditions, see section 2.2.3.

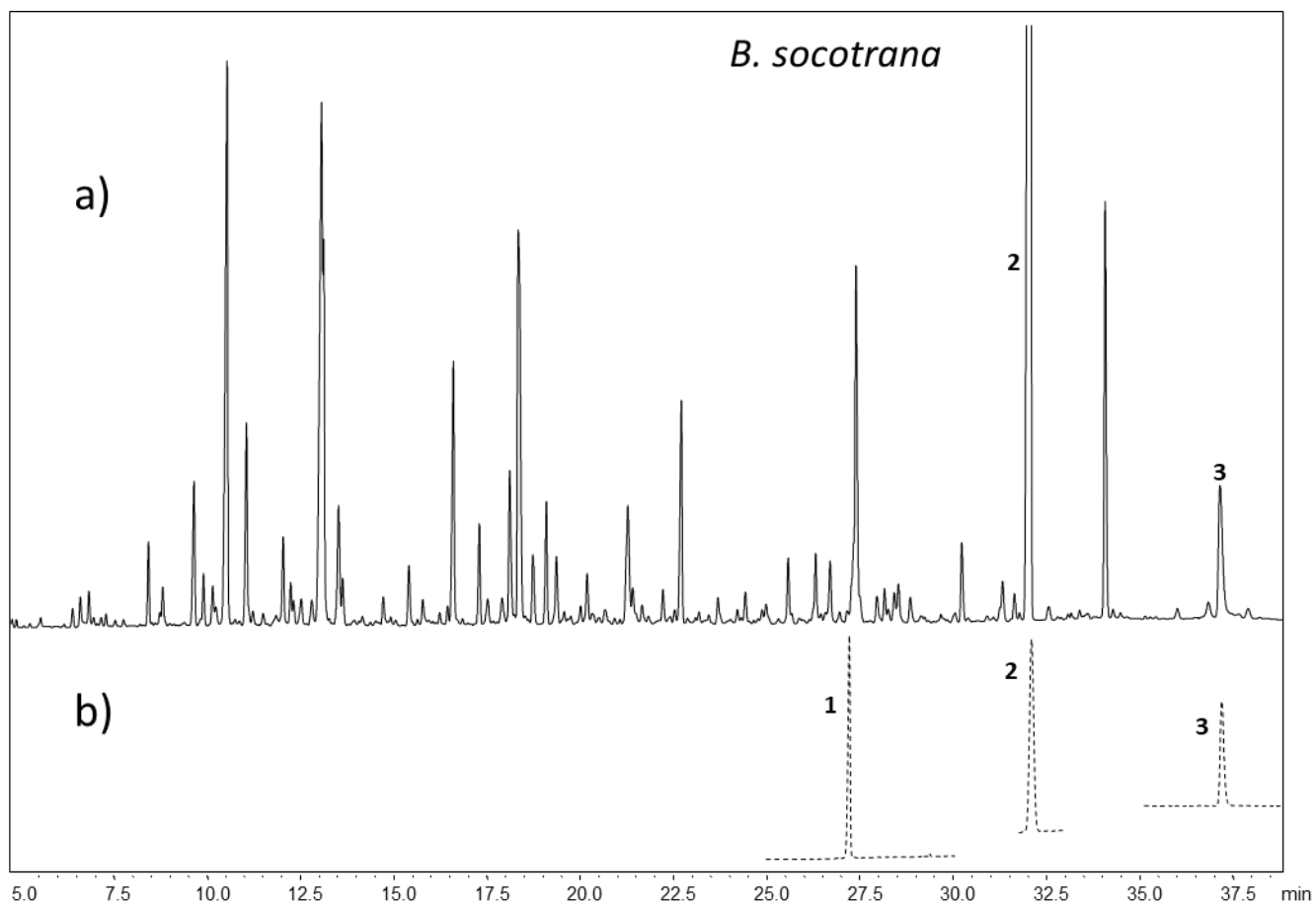
453 Peak identification: (Z,Z)-farnesol (1), (E,Z)-farnesol (2), (Z,E)-farnesol (3), (E,E)-farnesol (4), (Z)- $\alpha$ -santalol (5),  
454 (Z)- $\alpha$ -bergamotol (6), epi- $\beta$ -santalol (7), (Z)- $\beta$ -santalol (8), (E)- $\beta$ -santalol (9)



455

456

457 Figure 7: GC-FID patterns with column **IV** of a) frankincense sample of *Boswellia socotrana* EO, b) reference  
458 pure standards of incensyl acetate (**1**), serratol (**2**) and incensol (**3**). Analysis conditions: temp. progr.: 50°C  
459 (1min)//5°C/min//210°C (5 min) For the other analytical conditions see section 2.2.3.



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461

462

463 Table 1 – Dimensions and performance of the investigated [P<sub>66614</sub><sup>+</sup>] [Cl<sup>-</sup>] columns. Legend: L: length; *d<sub>c</sub>*: inner  
 464 diameter; *d<sub>f</sub>*: film thickness;  $\Delta s$ : separation number calculated between camphor (**7**) and borneol (**36**) of the  
 465 FFMIX; N: total number of theoretical plates measured on naphtalene; N/m: number of theoretical plates  
 466 per meter measured on naphtalene.

467

Columns						
N°	Dimensions			Characteristics		
	L (m)	<i>d<sub>c</sub></i> (mm)	<i>d<sub>f</sub></i> (μm)	$\Delta s$	N/m	N
<i>I</i>	30	0.25	0.15	731	2700	81000
<i>VI</i>	10	0.25	0.15	578	2700	27000
<i>VII</i>	5	0.25	0.15	507	2700	13500
<i>II</i>	20	0.18	0.12	816	4500	90000
<i>III</i>	10	0.10	0.06	928	6100	61000
<i>IV</i>	5	0.10	0.06	735	6100	30500
<i>V</i>	3	0.10	0.06	679	6100	18300