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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

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Abstract

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

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

1. Introduction

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

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

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

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

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

2. Experimental

2.1 Samples and chemicals

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

The following mixture or samples were used for this study:

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

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

iii) a Fatty Acid Methyl esters (FAMEs) standard solution from Merck consisting of 37 compounds dissolved 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, C16:1c, C17:0, C17:1c, C18:0, C18:1n9c, C18:1n9t, C18:2n6c, C18:2n6t, C18:3n6c, C18:3n3c, C20:0, C20:1n9c, C20:2n6c, C20:3n3c, C20:3n6c, C20:4n6c, C20:5n3c, C21:0, C22:0, C22:1n9c, C22:2n6c, C22:6n3c, C23:0, C24:0, and C24:1n9)

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

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

2.2 Analysis conditions

2.2.1. Instrumental set-up

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

2.2.2. Columns

The list of the $[P_{66614}^{+}][Cl^{-}]$ columns investigated together with their characteristics and performance are reported in Table 1. Columns with different characteristics were prepared by Mega (Legnano (MI), Italy) using the static coating procedure after a proprietary deactivation process of the fused silica surface.

2.2.3. GC-MS and GC-FID conditions

GC-MS analyses were carried out under the following conditions: temperatures: injector: 240°C; transfer line: 240°C, ion source: 200°C; carrier gas: He, flow control mode: constant linear velocity, flow rates: for 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 mm d_c) columns: 0.4 mL/min. Each column was connected to the MS through a post-column of deactivated 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 temperature higher than MAOT. Injection conditions were: mode: split; split ratio: 1:20 for columns ***I***. ***II***, ***VI***, ***VII*** and 1:50 for columns ***III***, ***IV***, ***V***, volume: Grob test: 2 µL, all other samples 1 µL. Oven temperature programs are reported in the captions of the corresponding figures.

GC-FID analyses were carried out under the following conditions: temperatures: injector: 240°C; detector: 240°C; carrier gas: H_2. Flow rates: 1 mL/min for conventional d_c columns, 0.7 mL/min for 0.18 mm d_c column, 0.4 mL/min for 0.10 mm d_c columns. All other analysis conditions were the same as those reported in the previous GC-MS paragraph. FID sampling rate: 40 ms. Oven temperature programs are reported in the captions of the corresponding figures.

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

2.2.4. Column characterization

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

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

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

3. RESULTS AND DISCUSSION

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

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

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

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

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

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

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

After these experiments, the mass loss after one hour was separately measured, because this time has been assumed as the maximum duration for the final isothermal step in a programmed temperature GC analysis 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% at 210°C and 0.2% at 220°C.

These results indicated that the MAOT affording good stability for columns coated with $[P_{66614}^+][Cl^-]$ can be fixed at 210°C. This value is also in agreement with the practical rule identified for IL GC stationary phases to consider as MAOT a temperature around 100°C lower than their decomposition temperature [13, 14]**.** A cycle 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 second column ***I*** after the usual conditioning at MAOT and resulted in a perfect overlapping of the 2,6-DMP retention times and a very stable baseline. Figure 1b reports the GC patterns of the Grob test after the injection n°1, n°10 and n° 20 and the enlargement of the 2,6-DMP (**8**) peak in these injections.

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

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

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

In this part of the study, the concurrent peculiar selectivity and high retention for oxygenated compounds of $[P_{66614}^+]$ $[Cl^-]$ has been exploited to lead these analytes to elute below column MAOT by acting on their dimensions.

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

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

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

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

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

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

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

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

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

3.2 Applications to real-world samples

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

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

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

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

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

4. Conclusions

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

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

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367 **References**

368 [1] M.J. Trujillo-Rodríguez, H. Nan, M. Varona, M.N. Emaus, I.D. Souza, J.L. Anderson, Advances of Ionic
369 Liquids in Analytical Chemistry, Anal Chem, 91 (2019) 505-531.
370 [2] D.W. Armstrong, L. He, Y.-S. Liu, Examination of Ionic Liquids and Their Interaction with Molecules, When
371 Used as Stationary Phases in Gas Chromatography, Anal Chem, 71 (1999) 3873-3876.
372 [3] C. Cagliero, C. Bicchi, Ionic liquids as gas chromatographic stationary phases: how can they change food
373 and natural-product analyses? , Anal Bioanal Chem, 412 (2020) 17-25.
374 [4] C. Cagliero, B. Sgorbini, C. Cordero, E. Liberto, C. Bicchi, P. Rubiolo, Analytical Strategies for Multipurpose
375 Studies of a Plant Volatile Fraction, in: K. Hostettmann, H. Stuppner, A. Marston, S. Chen (Eds.) Encyclopedia
376 of Analytical Chemistry, John Wiley & Sons, Ltd, 2014, pp. 447-466.
377 [5] P. Rubiolo, C. Cagliero, C. Cordero, E. Liberto, B. Sgorbini, C. Bicchi, Gas Chromatography in the Analysis
378 of Flavours and Fragrances, in: K. Dettmer-Wilde, W. Engewald (Eds.) Practical Gas Chromatography: A
379 Comprehensive Reference, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014, pp. 717-743.
380 [6] P. Rubiolo, E. Liberto, B. Sgorbini, R. Russo, J.L. Veuthey, C. Bicchi, Fast-GC-conventional quadrupole mass
381 spectrometry in essential oil analysis, J Sep Sci, 31 (2008) 1074-1084.
382 [7] Z.S. Breitbach, D.W. Armstrong, Characterization of phosphonium ionic liquids through a linear solvation
383 energy relationship and their use as GLC stationary phases, Anal Bioanal Chem, 390 (2008) 1605-1617.
384 [8] M. Mazzucotelli, C. Bicchi, A. Marengo, P. Rubiolo, S. Galli, J.L. Anderson, B. Sgorbini, C. Cagliero, Ionic
385 liquids as stationary phases for gas chromatography-Unusual selectivity of ionic liquids with a phosphonium
386 cation and different anions in the flavor, fragrance and essential oil analyses, J Chromatogr A, 1583 (2019)
387 124-135.
388 [9] K. Grob, G. Grob, K.J. Grob, Testing capillary gas chromatographic columns, J. Chromatogr., 219 (1981) 13-
389 20.
390 [10] K. Dettmer, Assessment of ionic liquid stationary phases for the GC analysis of fatty acid methyl esters,
391 Anal Bioanal Chem, 406 (2014) 4931-4939.
392 [11] L.M. Blumberg, Metrics of separation performance in chromatography: Part 2. Separation performance
393 of a heating ramp in temperature-programmed gas chromatography, J Chromatogr A, 1244 (2012) 148-160.
394 [12] C. Deferm, A. Van den Bossche, J. Luyten, H. Oosterhof, J. Fransaer, K. Binnemans, Thermal stability of
395 trihexyl(tetradecyl)phosphonium chloride, Phys Chem Chem Phys, 20 (2018) 2444-2456.
396 [13] R.A. Patil, M. Talebi, A. Berthod, D.W. Armstrong, Dicationic ionic liquid thermal decomposition
397 pathways, Anal Bioanal Chem, 410 (2018) 4645-4655.
398 [14] G.A. Odugbesi, H. Nan, M. Soltani, J.H. Davis, J.L. Anderson, Ultra-high thermal stability perarylated ionic
399 liquids as gas chromatographic stationary phases for the selective separation of polyaromatic hydrocarbons
400 and polychlorinated biphenyls, J Chromatogr A, 1604 (2019) 460466.

401 [15] M.S. Klee, L.M. Blumberg, Theoretical and practical aspects of fast gas chromatography and method
402 translation, J Chromatogr Sci, 40 (2002) 234-247.
403 [16] C. Bicchi, C. Brunelli, M. Galli, A. Sironi, Conventional inner diameter short capillary columns: an approach
404 to speeding up gas chromatographic analysis of medium complexity samples, J Chromatogr A, 931 (2001)
405 129-140.
406 [17] C. Bicchi, L. Blumberg, C. Cagliero, C. Cordero, P. Rubiolo, E. Liberto, Development of fast
407 enantioselective gas-chromatographic analysis using gas-chromatographic method-translation software in
408 routine essential oil analysis (lavender essential oil), J Chromatogr A, 1217 (2010) 1530-1536.
409 [18] C. Fanali, G. Micalizzi, P. Dugo, L. Mondello, Ionic liquids as stationary phases for fatty acid analysis by
410 gas chromatography, Analyst, 142 (2017) 4601-4612.
411 [19] N. Baldovini, C. Delasalle, D. Joulain, Phytochemistry of the heartwood from fragrant Santalum species:
412 a review, Flavour Frag. J., 26 (2011) 7-26.
413 [20] J. Niebler, A. Buettner, Identification of odorants in frankincense (Boswellia sacra Flueck.) by aroma
414 extract dilution analysis and two-dimensional gas chromatography-mass spectrometry/olfactometry,
415 Phytochemistry, 109 (2015) 66-75.

Captions to figures

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

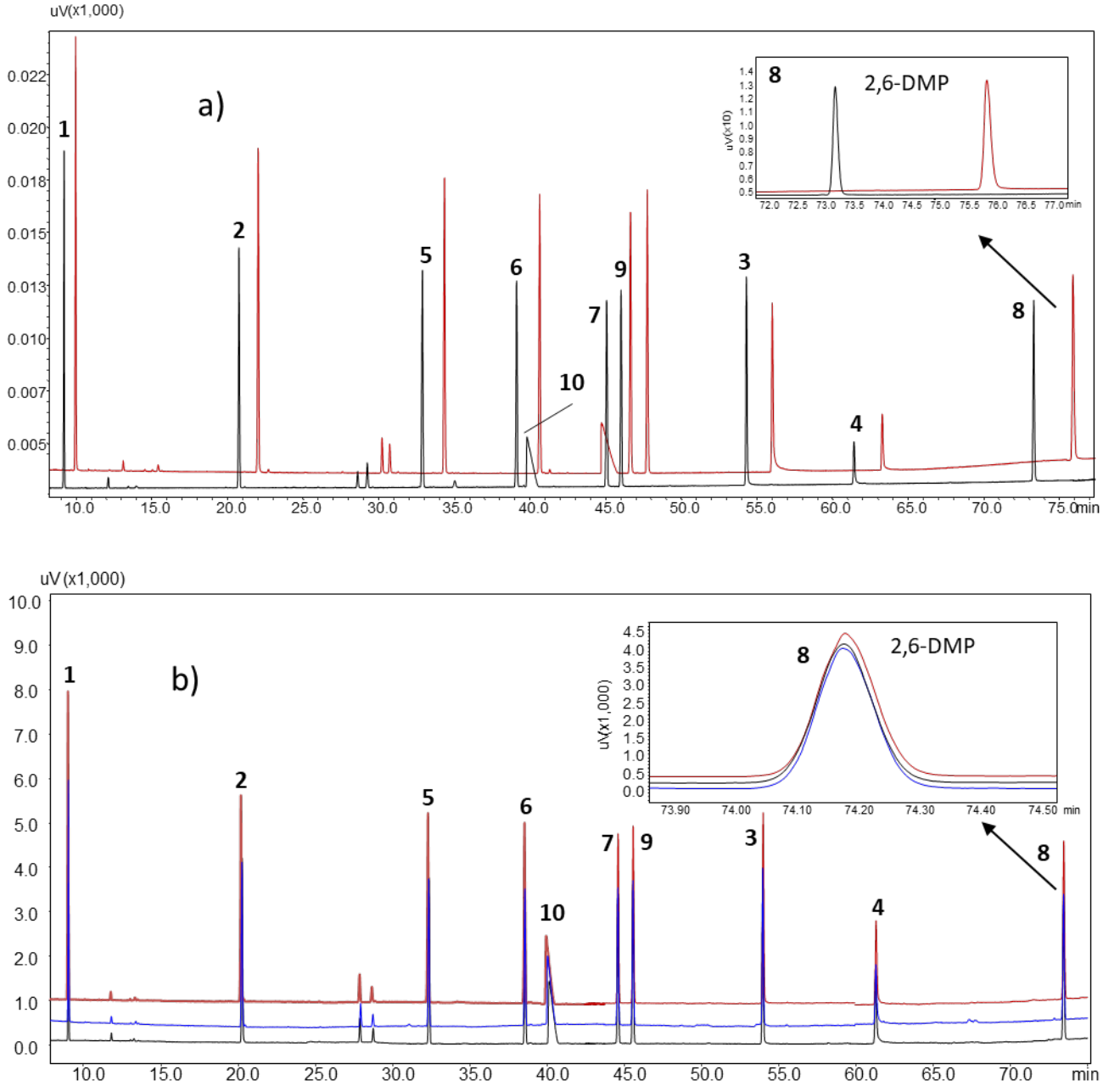


Figure 2: GC-FID patterns of peppermint essential oil analyzed on column ***I*** (a) and ***III*** (b)**.** Analysis conditions: temp. progr. Column ***I***: 50°C (1 min)//5°C/min//210°C (5 min); column ***III***: 50°C (1 min)//15°C/min//210°C (5 min). For the other analytical conditions, see section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

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

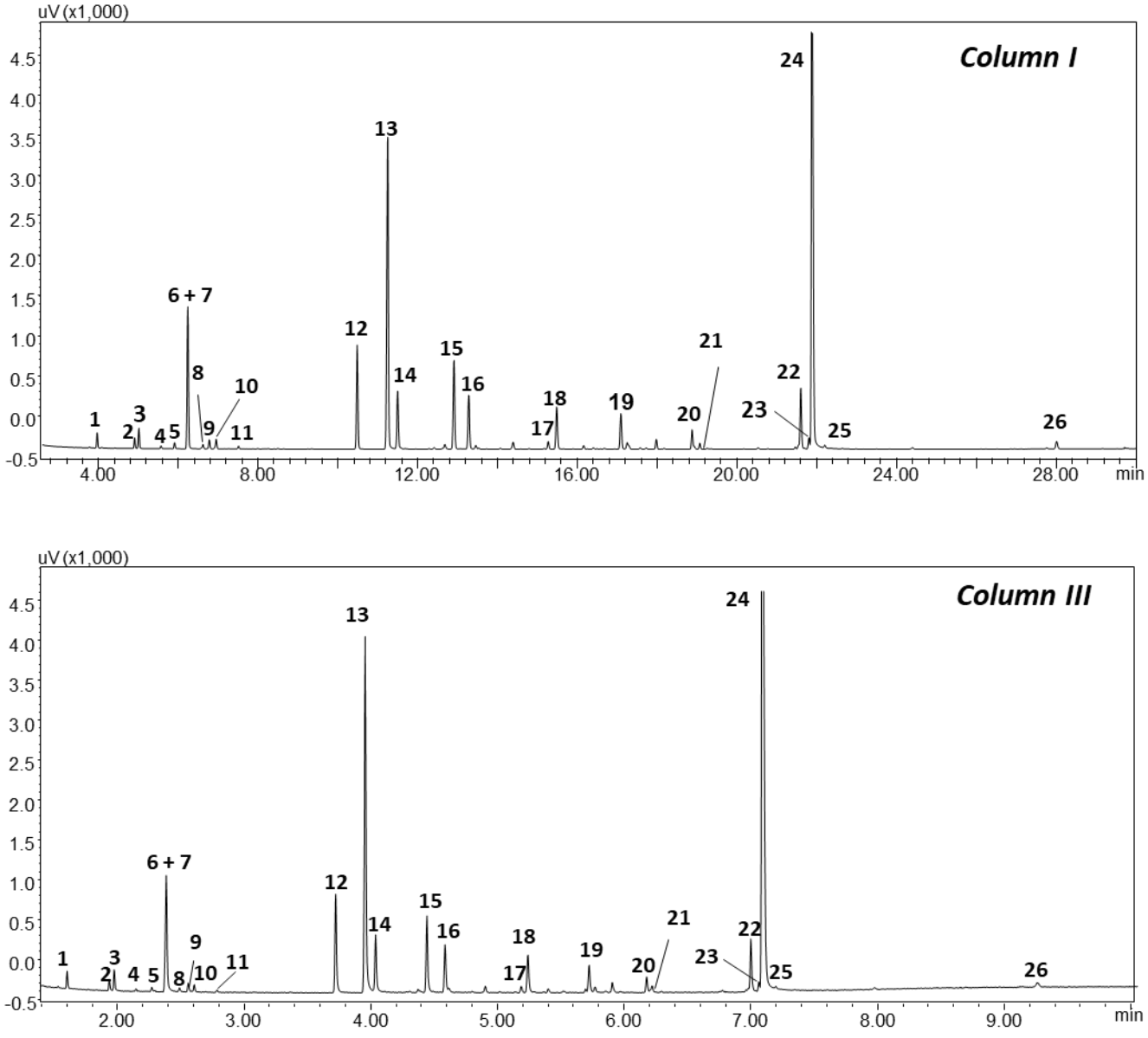


Figure 3: GC-FID patterns of FFMIX analyzed on the five $[P_{66614}^{+}][Cl^{-}]$columns investigated (Table 1). a) hydrocarbons, b) carbonyl derivatives, c) hydroxyl derivatives. Analysis conditions: temp. progr. Columns ***I*** and ***II***: 50°C (1min)//5°C/min/2/10°C (5 min); columns ***III***, ***IV***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

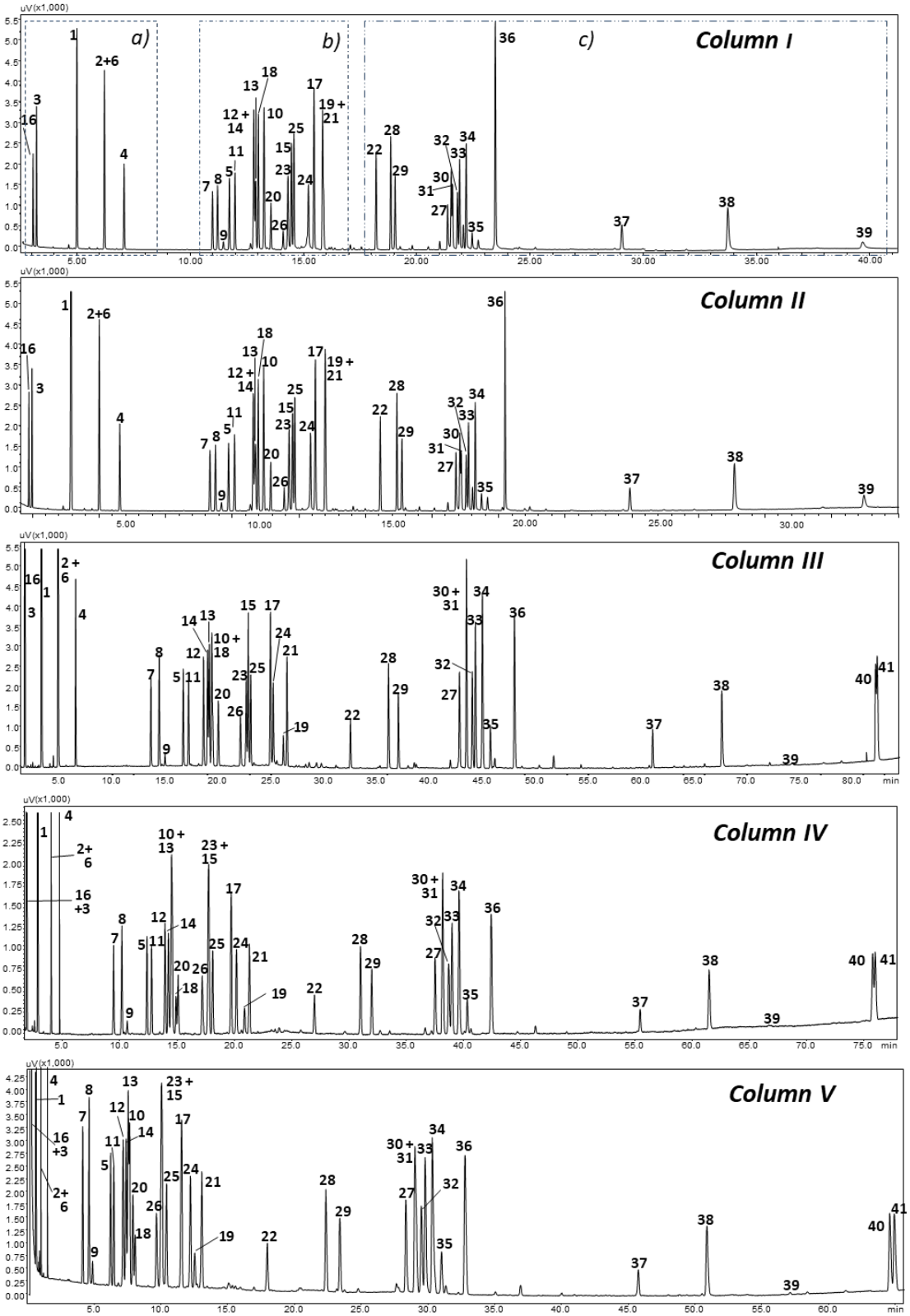


Figure 4: GC-FID patterns of oregano and thyme essential oil analyzed on column ***IV*.** Analysis conditions: temp. progr.: 50°C (1min)//5°C/min//210°C (5 min). For the other analytical conditions see section 2.2.3

Peak identification: β-myrcene (**1**), α-terpinene (**2**), α-pinene (**3**), *p*-cimene (**4**), γ-terpinene (**5**), camphor (**6**), *trans*-β-caryophyllene (**7**), terpinen-4-ol (**8**), linalool (**9**), borneol (**10**), carvacrol (**11**), thymol (**12**)

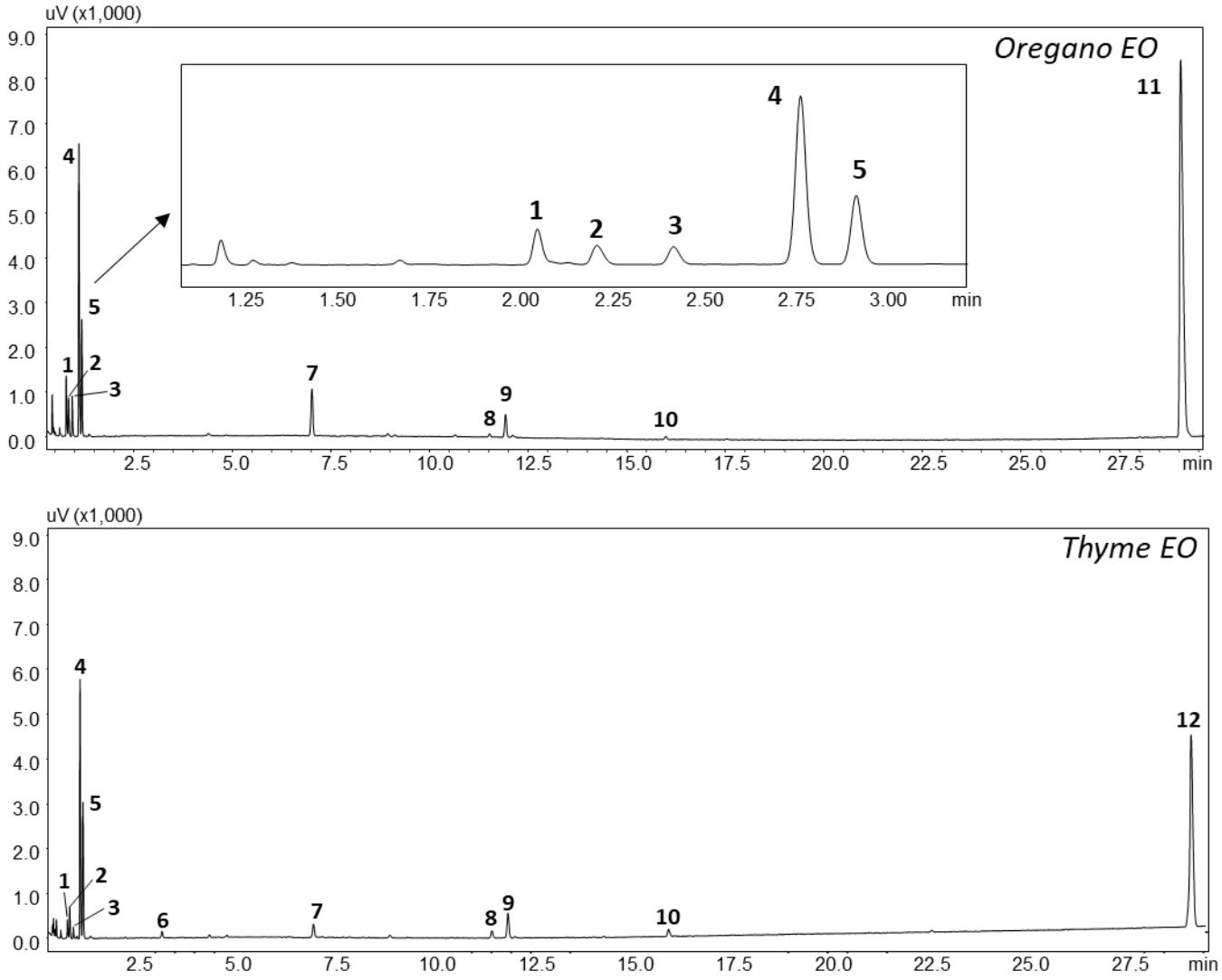


Figure 5: GC-FID patterns of the 37 FAME standard mixture analyzed with columns ***IV*** and ***V.***

Analysis conditions: temp. progr. Columns ***IV*** and ***V***: 50°C (1min)//2°C/min//210°C (5 min). For the other analytical conditions see section 2.2.3 and for cluster identification section 2.1.

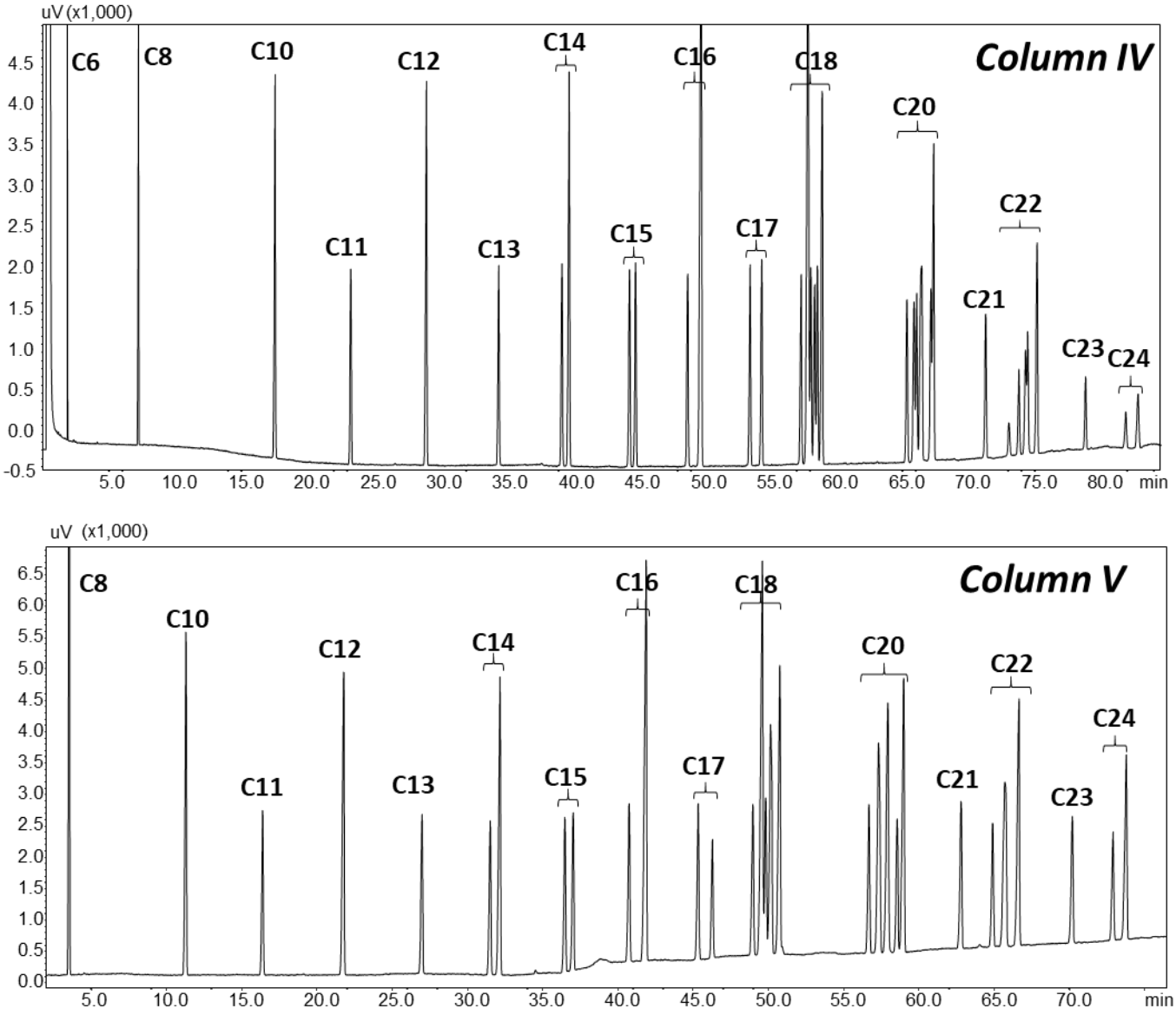


Figure 6: GC-FID patterns with column ***IV*** of *S. album* (**a**) and *S. yasi* EOs (**b**) and of the standard mixture of farnesols, santalols and bergamotol (**c**). Analysis conditions: temp. progr.: 50°C (1 min)//5°C/min//210°C (5 min). For the other analytical conditions, see section 2.2.3.

Peak identification: (*Z,Z*)-farnesol (**1**), (*E,Z*)-farnesol (**2**), (*Z,E*)-farnesol (**3**), (*E,E*)-farnesol (**4**), (*Z*)-α-santalol (**5**), (*Z*)-α-bergamotol (**6**), epi-β-santalol (**7**), (*Z*)-β-santalol, (**8**), (*E*)-β-santalol, (**9**)

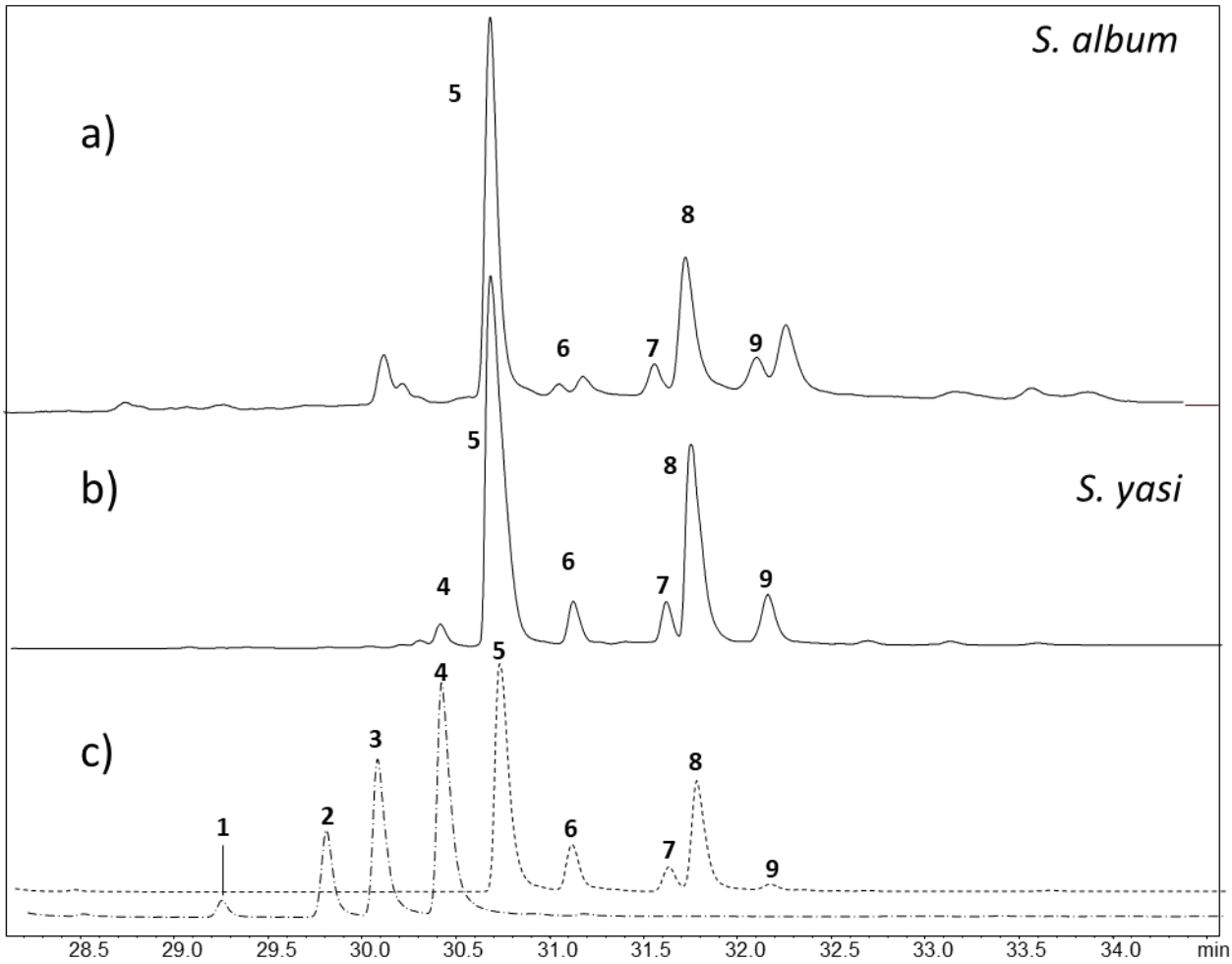
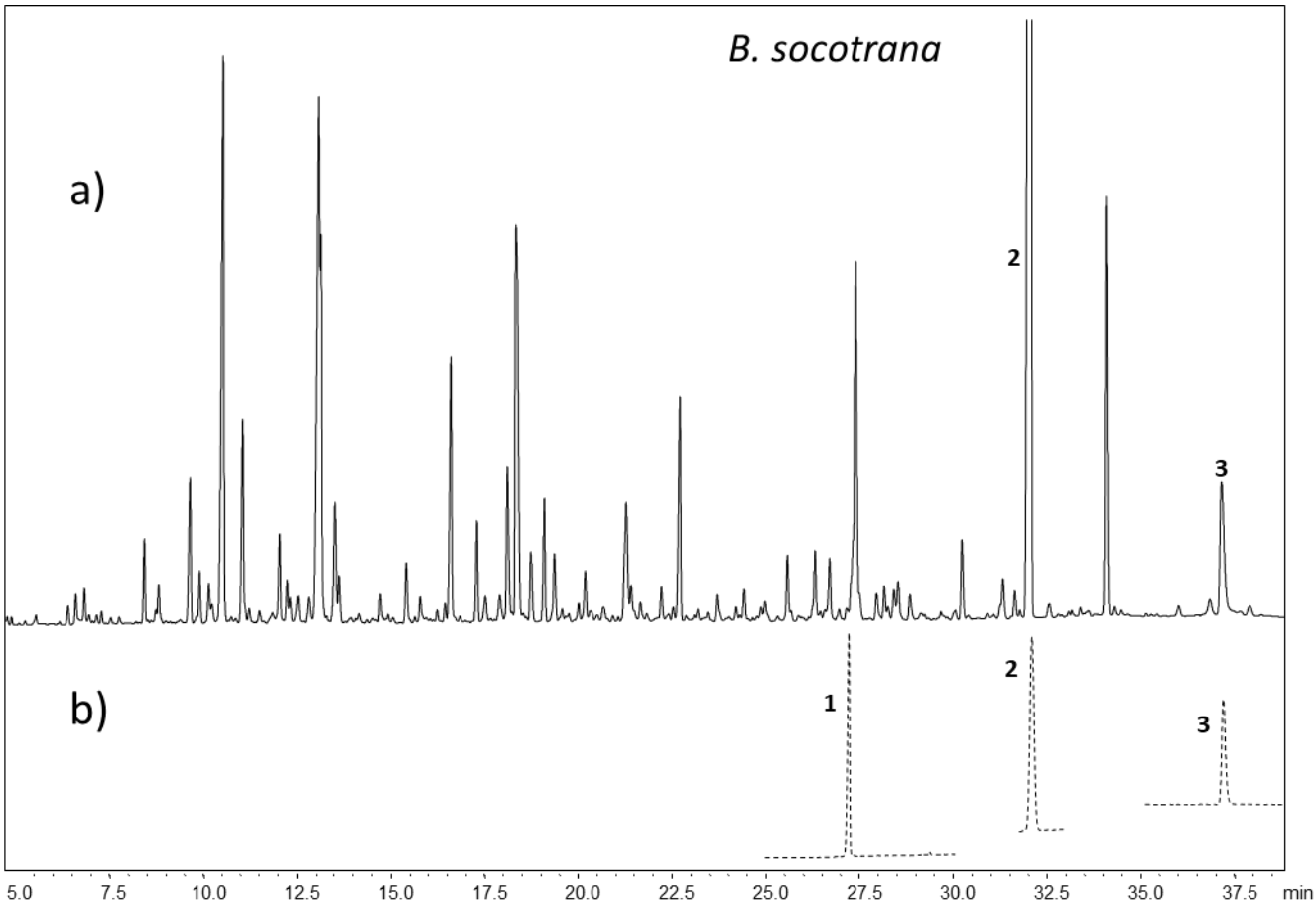


Figure 7: GC-FID patterns with column ***IV*** of a) frankincense sample of *Boswellia socotrana* EO, b) reference pure standards of incensyl acetate (**1**), serratol (**2**) and incensol (**3**). Analysis conditions: temp. progr.: 50°C (1min)//5°C/min//210°C (5 min) For the other analytical conditions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



463 Table 1 – Dimensions and performance of the investigated [P_{66614}^+] [Cl^-] columns. Legend: L: length; d_c: inner
464 diameter; d_f: film thickness; *Δs:* 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)	**d_c (mm)**	**d_f (µm)**	***Δs***	**N/m**	**N**
I	*30*	0.25	0.15	731	2700	81000
VI	*10*	0.25	0.15	578	2700	27000
VII	*5*	0.25	0.15	507	2700	13500
II	*20*	0.18	0.12	816	4500	90000
III	*10*	0.10	0.06	928	6100	61000
IV	*5*	0.10	0.06	735	6100	30500
V	*3*	0.10	0.06	679	6100	18300