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Room temperature ionic liquids as GC stationary phases in the analysis of fragrance of vegetable origin and essential oils. A new generation of highly inert GC columns

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Abstract
New GC stationary phases with different selectivity are continually investigated in the fragrance of vegetable origin and essential oil fields because of the sample complexity often consisting of mixtures of compounds with similar structural and physical characteristics (e.g. mono- and sesquiterpenoids), whose correct identification strongly depends on the synergic combination of chromatographic and mass spectral data. Ionic liquids (ILs) as GC stationary phases are of great interest in this field because of their selectivity that significantly differs from that of the currently applied phases and their high temperature stability.

A first generation of IL GC columns introduced some years ago showed to be competitive in terms of selectivity and efficiency with conventional columns (OV-1, OV-1701 and PEG-20M) when applied to these fields but presented a significant activity towards polar or active analytes, mainly affecting their quantitative analysis. A new generation of highly inactive columns coated with three of the most used ionic liquid GC stationary phases (IL 60i, IL 76i and IL 111i) has recently been introduced. The new inert IL columns have here carefully been tested mainly in view of their routine application in the fragrance and essential oil fields. The results showed that their selectivity is uncommon and perfectly comparable to that of the IL columns of the first generation, while efficiency and inertness is competitive with that of the currently used conventional columns. The IL column performances of both generation were compared through the quali-quantitative analysis of components in a group of different complexity samples, including the Grob test, a standard mixture of suspected allergens, and the essential oils of chamomile and sandalwood.

Keywords: Ionic liquids, GC stationary phases, selectivity, inertness, quali-quantitative analysis, suspected fragrance allergen, essential oils.
1. INTRODUCTION

Over the last decade, some room-temperature ionic liquids (ILs) have aroused considerable interest as stationary phases for gas chromatography. Their success is due to their unique and tunable selectivity depending on their chemical composition in combination with some general characteristics including their low volatility, high thermal stability (over 300°C), and negligible vapour pressure, and others more specific such as high viscosity and good wetting ability of the inner wall of fused silica capillaries. All these characteristics together make mono- and polycationic ILs, as well as Polymeric ILs (PILs), “good” stationary phases for conventional and multidimensional GC (MDGC). The IL possibility in analytical chemistry (i.e. in sample preparation and analysis) including GC have been extensively and exhaustively reviewed by Ho et al in 2014 [1]. This article also lists a number of other reviews describing developments, chromatographic properties and applications to specific fields of several ILs and PILs in GC and MDGC appeared over the past decade. Over the last two years, other reviews concerning IL applications in GC have been published by Hantao et al. [2], Kulsing et al. [3] and Sun et al. [4].

The ILs today available for GC mainly consist of an organic cation containing nitrogen or phosphorus (mainly phosphonium, imidazolium, pyridinium, and pyrrolidinium) combined with several inorganic or, more often, organic anions. Their selectivity is mainly due to their dual-nature retention mechanism, i.e. partition and interfacial adsorption or their combination [5-7] that widely extends the range of polarity of the analytes they are able to separate.

The first IL commercial column was introduced in 2008 with the acronym SLB-IL100: it was coated with poly[1,9-di(3-vinylimidazolium)-nonane] bis (trifluoromethyl) sulfonylimidate [NTf₂] and achieved a maximum operative temperature of 230°C [8, 9]. Other columns coated with different ILs (and thereby polarity and selectivity) were then made commercially available, namely SLB-IL59 (1,12-di-(tripropylphosphonium) dodecane [NTf₂]), SLB-IL60 (1,12-di(tripropylphosphonium) dodecane [NTf₂]), SLB-IL61 (a mixture of 1,12-di(tripropylphosphonium) dodecane paired with [NTf₂] and trifluoromethyl sulfonate), SLB-IL76 (tri-(tripropylphosphonium-hexanamido)-triethylamine [NTf₂]), SLB-IL82 (1,12-di(2,3-dimethyl-imidazolium) dodecane [NTf₂]), SLB-IL111 (1,5-di (2,3-dimethylimidazolium) pentane [NTf₂]). The number labelling the columns indicate their polarity and is determined by the sum of the Kovats retention indices normalized versus the polarity number of SLB-IL100 [10].

The peculiar selectivity makes ILs of high interest for the flavor, fragrance and essential oil (EO) fields, in general characterized by complex mixtures of isomeric components with similar structural and physical characteristics (e.g. mono- and sesquiterpenoids in EOs) that require the contribution of diagnostic chromatographic data (e.g. retention indices) besides mass spectra for a correct identification [11]. This field therefore constantly requires new stationary phases with unconventional selectivity combined with good chromatographic properties, to obtain separations
different from those achievable with the commonly-used polysiloxane and polyethylene glycol derivatives.

Some applications of IL columns have already been reported for the analysis of flavor and fragrance mixtures [12, 13], allergens [14] and some EOs (i.e. lemon essential oil [10] and fennel, cinnamon and nutmeg essential oils [15]).

Besides its contribution to the development of commercial IL columns, Mondello’s group applied ILs as stationary phase of the first GC column of a multidimesional GC-GC-GC preparative system, combined online to a LC pre-separation step operating in normal phase mode to isolate high purity minor components from essential oils at mg level. The system was applied to a very complex essential oil, i.e. vetiver essential oil, to isolate α-amorphene and β-vetivone. [16] and to sandalwood essential oil to isolate (Z)-α-santalol, (Z)-α-trans bergamotol, (Z)-β-santalol, epi-(Z)-β-santalol, α-bisabolol, (Z)-lanceol, and (Z)-nuciferol [17]. Wong et al. successfully applied IL columns as a second dimension in enantioselective-GC×GC to determine adulteration, or additives affecting the enantiomeric ratios, in commercial Australian tea tree oils[18].

In 2012, this research group systematically evaluated the performance of the commercially available SLB-IL59, SLB-IL60, SLB-IL61, SLB-IL76, SLB-IL82, SLB-IL100, SLB-IL111 columns in view of their routine application in the flavor, fragrance and EO fields and compared the results to those achieved with the conventional SE-52, OV-1701 and PEG-20M columns on a standard mixture of suspected allergens, commint and vetiver essential oils [19]. The study concluded that the investigated IL columns could competitively be applied to these fields, because of their performance and selectivity, but that further effort were still necessary in column manufacturing to achieve inertness comparable to that of conventional columns, i.e. to reduce their activity towards polar or active analytes, mainly for quantitative analysis, i.e. one of the main requirements to GC in this field.

As an answer to this generalized request, a new generation of highly inactive columns for three of the most popular ionic liquid GC stationary phases (IL-60i, IL-76i and IL-111i) has been introduced in 2016 to meet the request of inertness [20-22].

This study reports an evaluation of the performance of the new commercially-available inert IL columns, mainly in view of their routine use in the fragrance and essential oil fields. In particular, it compares the chromatographic performance and selectivity of the inert IL columns to those of the first generation and the possibility to apply them to quantitation in comparison to conventional columns. A standard mixture of suspected allergens and the essential oils of chamomile and sandalwood were used as samples of different complexity and composition representative of these fields.
2. EXPERIMENTAL

2.1 Samples and chemicals


The EOs of sandalwood (Santalum spicatum (R. Br.) A. DC), and chamomile (Matricaria chamomilla L.), were obtained by hydrodistillation following the European Pharmacopoeia [24] and were solubilized in cyclohexane at a concentration of 5 mg/ml before analysis. Solvents were all HPLC grade from Sigma–Aldrich (Milan, Italy).

2.2 Analysis conditions

Instrumental set-up: Analyses were carried out on a Shimadzu GC 2010 - Shimadzu QP2010-PLUS GC-MS system equipped with FID and elaborated with Shimadzu GCMS Solution 2.51 and GC Solution 2.53SU softwares (Shimadzu, Milan, Italy).

Columns: GC analyses were carried out with four 30 m × 0.25 mm d.c. × 0.25 μm d.f. conventional columns coated with 100% methyl-polysiloxane (OV-1) 95% methyl-polysiloxane 5%-phenyl (SE-
52), 7%-cyanopropyl, 7%-phenyl polysiloxane (OV-1701), and polyethylene glycol (PEG-20M) and six IL columns (30 m × 0.25 mm d.c. × 0.20 μm d.f.) of different polarity belonging to the two generation (i.e. SLB-IL60, SLB-IL76, SLB-IL111, and SLB-IL60i, SLB-IL76i, and SLB-IL111i). All IL columns were provided by Supelco (Bellefonte, PA, USA).

**GC-MS conditions:** Temperatures: injector: 250°C, transfer line: 207°C; ion source: 200°C; carrier gas: He, flow control mode: constant linear velocity; flow rate: 1mL/min. Injection mode: split; split ratio: 1:50, injection volume: 1µl. The MS operated in electron impact ionization mode (EI) at 70 eV, scan rate: 666 u/s, mass range: 35–350 m/z. FID temperature: 250°C, sampling rate: 40ms. Temperature programs: i) 40°C(1min)//2°C/min//250°C(2min) for the Grob test analyses, ii) 40°C(1min)//3°C/min//250°C(2min) for the allergens standard mixture and chamomile EO analyses, iii) 50°C(1min)//3°C/min//250°C(2min) for the santalol and farnesol standard mixtures and the sandalwood EO analyses (not optimized conditions), iv) 70°C(1min)//5°C/min//250°C(2min) for the santalol and farnesol standard mixtures and the sandalwood EO analyses (optimized conditions for IL-60i).

Analytes were identified by comparing their mass spectra and linear retention indices to those of authentic standards or to those of commercial and in-house libraries and literature. Retention indices were calculated versus a C9-C25 hydrocarbon solution analysed under the same conditions as reported above.

2.3 **Quantitation of farnesols and santalols in sandalwood essential oil**

Stock standard mixtures of farnesols and santalols were prepared by adding an aliquot of pure standard mixtures (see section 2.1) to an appropriate volume of cyclohexane at an initial concentration of 10 mg/mL. Suitable dilutions of each stock standard mixture in cyclohexane were then prepared in the concentration range (5-0.1 mg/ml) also adding bacdanol (CAS: 28219-61-6, purity 92.0%) at a concentration of 1 mg/ml as internal standard. The resulting solutions (stock and diluted) were stored at 0°C and renewed weekly. The external calibration method was applied to quantify the target components of the sandalwood essential oil with GC-FID by building a calibration curve for each compound. The analytical performances of the quantitation methods were tested in terms of analytes repeatability and intermediate precision, and R². Repeatability was measured by was analyzing in five times by GC-FID each point of the calibration curves while intermediate precision was measured by analyzing the same samples every three weeks over a period of three months. The sandalwood EO was diluted at 5 mg/ml in cyclohexane and analyzed using bacdanol (1 mg/ml) as internal standard. Chamomile EO was diluted at 5 mg/ml in cyclohexane and analyzed using pentadecane (1 mg/ml) as internal standard. These solutions were at the same time used for GC-FID analysis.
3. RESULTS AND DISCUSSION

The new generation of IL columns are characterized by a high inertness, thus overcoming one of the main weak points of the previous one (for short, from here called “conventional IL columns”), that severely counteracted their popularity for routine analysis in quality control laboratories.

In this section, the performance of the new inert IL columns is evaluated on the basis of their reliability and selectivity in routine work mainly in view of their application to quantitative analysis in the fragrance and essential oil fields when used for a standard mixtures of suspected allergens, and essential oils of chamomile, and sandalwood. The results are compared to those obtained with the currently used columns, namely SE-52, OV1, OV-1701 and PEG-20M taken as references.

One of the first evidence of the better inertness achieved by the new IL columns was with GC-MS analyses. Quantitative analyses with conventional IL columns had better be run with GC-FID because of “erratic” analyte adsorptions affecting peak areas due to the high temperatures of the GC-MS transfer-line. The analyte adsorption was also difficult to standardize since it varied depending on analyte polarity and organic functions, and analysis conditions. This disadvantage could be avoided by adopting FID detection, or partially overcome by decreasing the transfer-line temperature below 200°C, which, however, could interfere with a consistent quantitative elution of the low-volatility components. This limit has successfully been overcome by the new inert IL columns.

3.1 Inertness of the new IL columns

Inertness was first evaluated with the Grob test and a standard mixture of the 29 suspected allergens regulated by the European Commission, i.e. compounds that belong to different chemical classes and cover a wide range of volatility and polarity [25]. The performances of the three new columns (IL-60i, IL-76i and IL-111i) were compared to those of the corresponding conventional ones (IL-60, IL-76 and IL-111), taking OV-1701 as reference.

The parameters investigated with the Grob test were average tailing factors, peak width (σ) and number of asymmetric peaks. Figure 1 reports the Grob test GC-MS patterns of the investigated columns and Table 1 the data concerning the parameters characteristic of the columns.

The Grob test results shows that the average peak width (σ) for the inert IL columns were similar, although slightly higher than to those of OV-1701 (from 1.13 (IL-60i) to 1.45 (IL-76i) times higher) and in general better than those of the conventional IL columns, whose average σ was between two (IL-60) and three (IL-76) times higher than OV-1701. The number of asymmetrical peaks (i.e. peaks with tailing factors above 1.1 or below 0.9) is drastically decreased compared to the conventional IL columns reaching a minimum of three on the nine peaks of the Grob test for the inert IL columns, exactly as for the OV-1701 reference. A similar consideration can be done on the average tailing factor that resulted even better than that of OV-1701 for IL-60i (1.024 vs. 1.017), drastically improved for IL-76i compared to IL-76 (1.050 and 1.533 respectively), while it is only...
slightly ameliorated for IL-111i vs. IL-111. These results testify the increased inertness achieved by the inert IL columns.

Similar, if not better results were obtained with the allergens standard mixture. Figure 2 compares its GC-FID profiles obtained with OV-1701, IL-60 and IL60i columns. The column performances are reported in Table 2 that also includes the number of adsorbed peaks (i.e. peaks with percentage areas reduction vs. their areas on OV-1701 below 90%) and the separation measure (Δs), (i.e. the number of consecutive non-overlapping σ-intervals within an arbitrary time interval (t_b – t_a) [26]. IL-60i shows the best average tailing factor being it very close to the theoretical value (1.002); average peak width is very similar to that of OV-1701, and both the numbers of asymmetric and adsorbed peaks are about the half of those of IL-60. Figure 3 reports the adsorptions relative to OV1701 of the components of the allergens standard mixture with inert and conventional IL-60 columns. This diagram show that, with IL 60i, most compounds present areas vs. OV-1701 above 80 %; only anisyl alcohol (18) and vanillin (24) achieve 75% and 65% respectively, at the same time some of them seems to be less adsorbed than on OV-1701, in particular, eugenol (12), methyl eugenol (15), hydroxycitronellal (13), and benzyl salicylate (28). The adsorption rate get drastically worse with IL-60, where the intensity of several peaks is seriously affected by the adsorption phenomenon. The separation number Δs of IL-60i is decidedly higher than that of OV-1701 and, to a lesser extent, of IL-60, meaning that its chromatographic properties together with its better selectivity improve column performance [27].

The next paragraph discusses more in details the results of the allergens standard mixture when analysed by GC-MS with IL-60 and IL-60i columns. Figure 4 reports three examples emphasizing the influence of inertness on the GC-MS patterns of the allergen standard mixture with the two IL-60 columns. Phenylacetaldehyde (4) and methyl oct-2-ynoate (5) (figure 4A) behave differently since they are both adsorbed on IL-60 (although differently, 10% and 95% respectively); moreover, the peak of phenylacetaldehyde is asymmetric (tailing factor 1.374) and also very large (σ: 0.121 min); with the IL-60i the two peaks present a high degree of symmetry and a null, or very limited adsorption, versus OV-1701. The next example (Figure 4B) concerns two of the three farnesol isomers (19a,19b), p-anisyl alcohol (18) and cynnamyl alcohol (20) that are almost non-detectable with the IL-60 column, while, again, their areas are little or nearly unchanged with IL-60i. The two farnesol isomers are discussed in detail in the next paragraph while the adsorption of p-anisyl alcohol varies from 85% with IL60 to 25% with IL 60i and that of cynnamyl alcohol from 52% with IL60 to 20% with IL 60i. The last example (Figure 4C) refers α-hexyl cynamaldehyde (22) and α-pentyl-cynamal alcohol (23): the former gained 14% in intensity with IL-60i compared to IL-60 because of the lower adsorption, while the latter is almost completely missed with IL-60 (91% of adsorption) is little adsorbed with IL-60i (20%). Similar, but less evident; results were obtained with IL76i and IL111i stationary phases.
3.2 IL column inertness and sample classification (chemotype definition)

This example concerns how inertness can influence quality control and characterization of a natural product. Chamomile flower-heads (*Matricaria recutita* L.) are widely used in traditional medicine for its well-known spasmolitic, sedative, anti-inflammatory and antiseptic effects [28]. Their quality and commercial value in general depend on the contents of EO (mainly sesquiterpenoids) and flavonoids (mainly apigenine and related glucosides) [28].

The chamomile EO obtained by hydrodistillation [24] is mainly characterized by a low number of sesquiterpenoids, from which most abundant component(s), Schilcher defined six different chemotypes: A) α-bisabolol oxide A (9); B) α-bisabolol oxide B (5), C) α-bisabolol (6), D) α-bisabolol (6) and α-bisabolol oxides A (9) and B (5) in comparable amounts, E) α-bisabolone oxide A (7), and F) green EO from chamomile flower heads containing low amount of matricine [29, 30].

This application exemplifies how column inertness can affect a correct definition of the chemotype of a sample of chamomile, and, thereby, of its commercial value. The usual approach to characterize a sample quantitatively in the fragrance and EO fields is the percentage normalization of the areas vs. an internal standard (here C15) with GC-FID and FID response factors [31, 32].

Figure 5 shows the GC-FID patterns of the chamomile EO carried out on a) IL-111 and b) IL-111i columns. The results are compared to those carried out on a SE-52 conventional column taken as reference. The normalized percentages on IL-111 determined vs. an internal standard (C15) significantly differs from those of IL-111i, which, however, are within the same range of those obtained with SE-52. This difference makes difficult an unequivocal attribution of the sample to a chemotype. From the results obtained with IL-111, the sample seems to belong to the chemotype D (α-bisabolol (6) and α-bisabolol oxide A (9) and B (5)), while those with IL-111i are compatible with the chemotype A (α-bisabolol oxide A (9)). Table 3 reports percentage abundance and percentage adsorption of the target components calculated vs. those obtained with SE-52 taken as 100% in *Matricaria chamomilla* L. EO. The results show that the three peaks characteristic of chemotype D are strongly adsorbed and at a different extent with the two IL columns. With IL-111 adsorption ranged from about 60% with α-bisabolol oxide A to about 20% of α-bisabolol oxide B and α-bisabolol, thus fully altering the ratio between the peaks and, in consequence, affecting a correct identification of the chemotype. On the other hand, IL-111i shows very low percentages of adsorption compared to those of SE-52, thus assessing that the investigated sample belongs to the chemotype A (α-bisabolol oxide A). These results are confirmed by those obtained with the conventional SE-52 column that is often adopted for this analysis.

3.3 Inert IL columns and quantitative analysis
This paragraph deals with the importance of inertness on the definition of the quality of an EO of high commercial value. *Santalum* species (Santalaceae) and their EOs still are a primary natural raw material for the perfume industry despite shortages and price rises of *Santalum album* EO, and the availability of cheap synthetic sandalwood odorants. Commercial sandalwood EOs are obtained by steam distillation of the heartwood of 20-year-old trees, mainly from three *Santalum* species: a) *Santalum album* L., also known as ‘East Indian’, b) *S. austrocaledonicum* Vieill., c) *S. spicatum* (R. Br.) A. DC. Other *Santalum* species involved with commercial sandalwood EOs include *S. paniculatum* Hook. & Arn. and *S. insulare* Bertero ex A.DC, as well as species from other genera such as *Osyris tenuifolia* Engl.. Sandalwood EOs and thereby their organoleptic properties, quality and commercial value are characterized by an informative sesquiterpenoid fraction; in particular, the qual-quantitative profile of farnesol and santalol isomers is diagnostic of botanical and geographical origin and quality [33]. Quality characteristics (QC) of *S. album* and *S. spicatum* EOs are regulated by international norms in particular ISO 3518 (2002) [34] [for *S. album* and ISO 22769 (2009) [35] for *S. spicatum*. Moreover, EU regulatory rules on suspected volatile fragrance allergens require that (E,E)-farnesol (a minor component characteristic of *S. spicatum*) is quantified [200]; and the same will be for α- and β-santalols with the forthcoming list. Unfortunately, these alcohols are not base-line separated in a single GC run with conventional stationary phases, since with non-polar columns, two of the four isomers of farnesol co-elute (i.e. the (E,Z) and the (Z,E) isomers), the (E,E)-isomer co-elutes with nuciferol, and Z-trans-α-bergamotol coelutes with a bisabolol isomer, and, with polar columns, (E,E)-farnesol almost fully overlap with Z-trans-α-bergamotol. A correct quantitative analysis therefore requires the application of either a double run on two conventional stationary phases or a multidimensional GC method (GCxGC or MDGC) [17, 36].

Due to their peculiar selectivity, ionic liquids have therefore been tested as stationary phases to separate simultaneously the four santalol and the four farnesol isomers in a single GC run. Figure 6 shows the GC separations of the santalol and farnesol standard mixtures with a) OV1, b) PEG-20M, c) IL-60 and d) IL-60i columns as such and optimized (e). IL-60 and IL-60i provided a base line separation of all eight isomers, while IL-76 and IL-76i and IL-111 and IL111i showed some partial peak overlapping (data not shown). On the other hand, IL-60 produce some peak distortion and analyte adsorption as shown by Figure 7, which reports adsorption rates (a) and tailing factors (b) of the eight sesquiterpene alcohols when analyzed by GC-FID with IL-60 and IL-60i and adopting PEG-20M as reference column. These diagrams indicate that a) analyte adsorptions with IL-60 are of about 15% for most components while the maximum achieved with IL-60i is around 5% for epi-β-santalol, and b) the analyte tailing factors with IL-60 are all around 1.2 while those of IL-60i are almost the same as those of PEG-20M, and very close to 1.0 thus making easier peak integrations. Moreover, IL-60 can only be used with GC-FID and not with GC-MS analysis (see
above) limiting the analytical potential to define quality and discover adulterations and making retention indices indispensable for a correct peak location in the GC-FID chromatogram.

S. spicatum of Australian origin is of high interest for the perfume industry and it is characterized by the following quality markers and concentration ranges: (Z)-α-santalol (15-25%), (Z)-β-santalol (5-20%), epi-β-santalol (0.5-3.5%), (Z)-α-trans-bergamotol (2-10%) and (E,E)-farnesol (2.5-15%) [37].

A S. spicatum EO from Australia declared as authentic is here qual-quantitatively investigated to confirm its quality and origin. In this case too, the percentage normalization of the areas vs. an internal standard (badanol) with GC-FID and FID response factors was first applied. [31, 32].

Figure 8 shows the GC-FID profiles of the Australian S. spicatum EO with a) OV-1, b) PEG-20M, c) IL-60, and d) IL60i columns. The results show that some markers overlaps or coelute with OV-1 and PEG-20M while all of them are baseline separated with IL-60 and IL-60i. Table 4 reports normalized area percentages of the investigated markers determined by GC-FID and shows that it is possible to determine accurately the sample composition in a single run with the IL columns and state that the investigated S. spicatum EO is authentic and of Australian origin. The same approach cannot be used for the OV1 and PEG-20M columns because of the alteration of area percentage of some markers due to partial or full coelution with other components (i.e. (Z)-α-trans-bergamotol and α-bisabolol isomer, (Z)-β santalol and an unknown, and, (E,E)-farnesol and nuciferol with OV1 column, and (Z)-α-trans-bergamotol) and (E,E)-farnesol with PEG-20M).

The same EO sample was also submitted to true quantitation to detect, although indirectly, possible adulteration with cheaper ‘heavy’ diluents (e.g. polyethyleneglycol, coconut oil, castor oil or cedarwood oil) not eluting in GC, and therefore not altering the marker ratios and percentages in the final results. True quantitation was carried out by external calibration with the pure standards; these analyses were also used to evaluate analytical performances in terms of analyte repeatability and intermediate precision and $R^2$ to assess consistency of column behavior over time. The $R^2$ values shows that linearity in the concentration range investigated of IL columns is very good. Also repeatability is very good with both the IL columns while intermediate precision, that for the IL60 is slightly lower than OV1 and PEG-20M, is very satisfactory for IL60i thus assessing the stability of column inertness and performance (Table 5). The quantitative results confirm that the EO investigated was authentic and not adulterated with “heavy” oils. The true quantitation of farnesols and santalols is also necessary when sandalwood EO enter in perfume compositions or cosmetic formulations to control if their amount is within the thresholds fixed by the EU list of suspected volatile fragrance allergens. The quali-quantitative characterization of sandalwood EOs from different species and origin with ILs as GC stationary phases is the object of a forthcoming publication.

4. Conclusions
The above results show that the new generation of columns coated with IL stationary phases are in all competitive with those currently used (e.g. SE-52, OV-1, OV-1701 and PEG-20M) for qualitative analysis in the fragrance and EO fields. The new generation combines the unusual selectivity of ionic liquids to the efficiency and inertness deriving from a new proprietary deactivation process. These characteristics produce peaks whose shape is highly symmetric with areas that are independent on the analyte structure and functionality, and enables to apply reliably the percentage normalization and true quantitation methods to characterize samples in this field, at the same time assuring high repeatability and intermediate precision.

Reliable IL columns with performance suitable for routine analysis in quality control offer to these fields new tools to carry out a number of applications in a single run with routine instrumentation, and avoid to adopt more complex and expensive approaches such as multidimensional GC or more time consuming multiple injection in two or more conventional columns. Moreover, the interest of ILs as GC stationary phases in the routine of these fields is expected to increase furtherly thanks to the recent introduction of water compatible columns, that afford to inject directly aqueous or hydroalcoholic samples avoiding time consuming sample preparation steps.

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[37] AS 2112 - 2003 - Oil of sandalwood [*Santalum spicatum* (R.Br.) A.DC.].
Captions to figures

Figure 1: GC-MS patterns of the Grob test standard mixture with the columns investigated. Analysis conditions: temperature program: 40°C(1min)//2°C/min//250°C(2min), flow rate: 1 ml/min. Peak identification: 1: decane, 2: undecane, 3: dodecane, 4: 1-octanol, 5: methyl decanoate, 6: methyl undecanoate, 7: methyl dodecanoate, 8: 2,6-dimethylphenol, 9: 2,6-dimethylaniline, 10: dicyclohexylamine, 11: 2-ethylcaproic acid

Figure 2: GC-FID patterns of the allergens standard mixture with OV-1701, IL60 and IL 60i columns. Analysis conditions: temperature program: 40°C(1min)//3°C/min//250°C(2min), flow rate: 1 ml/min. Peak identification: see Section 2.1.

Figure 3: Adsorption of suspected allergens, calculated from the absolute area ratio of each compound with each investigated IL versus OV-1701 columns, taken as reference.

Figure 4: GC-MS patterns (a and b) and relative enlargements (c-h) of the allergens standard mixture with IL60 and IL 60i columns. Analysis conditions: temperature program: 40°C(1min)//3°C/min//250°C(2min), flow rate: 1 ml/min. Peak identification: see Section 2.1.

Figure 5: GC-FID patterns of santalol (——) and farnesol (——) standard mixture with a) OV-1, b) PEG-20M, c) IL60, d) IL60i, e) IL60i (optimized method) columns. Analysis conditions: (a-d) temperature program: 50°C(1min)//3°C/min//250°C(2min), (e) temperature program:70°C(1min)//5°C/min//250°C(2min); flow rate: 1 ml/min. Peak identification: 1: (Z,Z)-farnesol, 2: (E,Z)-farnesol, 3: (Z,E)-farnesol, 4: (E,E)-farnesol, 5: (Z)-α santalol, 6: (Z)-α- trans-bergamotol, 7:epi-β santalol, 8: (Z)-β santalol.

Figure 6: adsorption (a) and tailing factor (b) of santalols and farnesols standard mixtures; adsorption was calculated from the absolute area ratio of each compound with each investigated IL versus PEG-20M columns, taken as reference.

Figure 7: GC-FID patterns of sandalwood (Santalum spicatum (R. Br.) A. DC) essential oil with a) OV1, b) PEG-20M, c) IL60, d) IL60i (optimized method) columns. Analysis conditions: (a-c) temperature program: 50°C(1min)//3°C/min//250°C(2min), (d) temperature program:70°C(1min)//5°C/min//250°C(2min), flow rate: 1 ml/min. Peak identification: 1: (Z,Z)-farnesol, 2: (E,Z)-farnesol, 3: (Z,E)-farnesol, 4: (E,E)-farnesol, 5: (Z)-α santalol, 6: (Z)-α-trans-bergamotol, 7:epi-β santalol, 8: (Z)-β santalol, 9: bisabolol isomer, 10: nuciferol, ISTD: baccanal.

Figure 8: GC-FID patterns of chamomile (Matricaria chamomilla L.) essential oil with IL-111 and IL-111i columns. Analysis conditions: temperature program: 40°C (1min)//3°C/min//250°C(2min), flow
Table 1: Average peak width ($\sigma$), average tailing factor, number (#) of asymmetric peaks, for the Grob test standard mixture with the columns investigated.

<table>
<thead>
<tr>
<th>Column</th>
<th>Average tailing factor</th>
<th>Average $\sigma$ (min)</th>
<th># asymmetric peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1701</td>
<td>1.024</td>
<td>0.029</td>
<td>3</td>
</tr>
<tr>
<td>IL-60</td>
<td>1.030</td>
<td>0.059</td>
<td>6</td>
</tr>
<tr>
<td>IL-60i</td>
<td>1.017</td>
<td>0.033</td>
<td>3</td>
</tr>
<tr>
<td>IL-76</td>
<td>1.533</td>
<td>0.087</td>
<td>4</td>
</tr>
<tr>
<td>IL-76i</td>
<td>1.050</td>
<td>0.042</td>
<td>3</td>
</tr>
<tr>
<td>IL-111</td>
<td>1.198</td>
<td>0.069</td>
<td>6</td>
</tr>
<tr>
<td>IL-111i</td>
<td>1.187</td>
<td>0.040</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2: Average peak width (σ), average tailing factor, number (#) of asymmetric peaks, number (#) of adsorbed peaks and separation measure (Δs) for the allergen standard mixture with the columns investigated. For adsorbed peak evaluation, see text.

<table>
<thead>
<tr>
<th></th>
<th>Average tailing factor</th>
<th>Average σ (min)</th>
<th># asymmetric peaks</th>
<th># adsorbed peaks</th>
<th>Δs</th>
</tr>
</thead>
<tbody>
<tr>
<td>OV-1701</td>
<td>0.917</td>
<td>0.031</td>
<td>2</td>
<td></td>
<td>1582</td>
</tr>
<tr>
<td>IL-60</td>
<td>0.993</td>
<td>0.038</td>
<td>10</td>
<td>13</td>
<td>1744</td>
</tr>
<tr>
<td>IL-60i</td>
<td>1.002</td>
<td>0.035</td>
<td>6</td>
<td>7</td>
<td>1868</td>
</tr>
</tbody>
</table>
Table 3: percentage abundance and percentage adsorption of the target components in *Matricaria chamomilla* L essential oil

<table>
<thead>
<tr>
<th></th>
<th>% abundance</th>
<th></th>
<th></th>
<th>% adsorption</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE-52</td>
<td>IL-111i</td>
<td>IL-111</td>
<td>IL-111i</td>
<td>IL-111</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(E)-β-farnesene</td>
<td>23.0</td>
<td>22.1</td>
<td>34.8</td>
<td>-0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>Germacrene D</td>
<td>2.1</td>
<td>1.8</td>
<td>2.7</td>
<td>0.0</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td>α-Farnesene</td>
<td>1.1</td>
<td>1.2</td>
<td>0.3</td>
<td>0.2</td>
<td>81.5</td>
</tr>
<tr>
<td>4</td>
<td>Bicyclogermacrene</td>
<td>1.6</td>
<td>1.4</td>
<td>2.3</td>
<td>0.0</td>
<td>-0.2</td>
</tr>
<tr>
<td>5</td>
<td>α-Bisabolol oxide B</td>
<td>9.0</td>
<td>9.8</td>
<td>12.4</td>
<td>0.3</td>
<td>19.2</td>
</tr>
<tr>
<td>6</td>
<td>α-Bisabolol</td>
<td>2.3</td>
<td>2.2</td>
<td>2.8</td>
<td>0.6</td>
<td>20.0</td>
</tr>
<tr>
<td>7</td>
<td>α-Bisabolone oxide A</td>
<td>6.8</td>
<td>6.7</td>
<td>5.3</td>
<td>0.1</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>α-Bisabolol oxide A</td>
<td>47.3</td>
<td>48.7</td>
<td>30.9</td>
<td>0.2</td>
<td>59.7</td>
</tr>
<tr>
<td>9</td>
<td>Chamazulene</td>
<td>3.0</td>
<td>2.8</td>
<td>4.5</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>Spiroether</td>
<td>3.8</td>
<td>3.2</td>
<td>4.0</td>
<td>0.5</td>
<td>21.9</td>
</tr>
</tbody>
</table>
Table 4: percentage abundance and absolute amount of the target components in *Santalum spicatum* (R. Br.) A. DC essential oil

<table>
<thead>
<tr>
<th></th>
<th>% abundance</th>
<th>Absolute amount (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEG</td>
<td>OV-1</td>
</tr>
<tr>
<td><em>(E,E)</em>-farnesol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.7</td>
<td>13.6</td>
</tr>
<tr>
<td><em>(Z)</em>-α santalol</td>
<td>17.9</td>
<td>18.2</td>
</tr>
<tr>
<td><em>(Z)</em>-α-trans-bergamotol</td>
<td>2.4</td>
<td>12.3</td>
</tr>
<tr>
<td>epi-β santalol</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td><em>(Z)</em>-β santalol</td>
<td>5.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Table 5: figures of merit of the different columns investigated for the quantification of santalols and farnesols in sandalwood essential oil

<table>
<thead>
<tr>
<th>N</th>
<th>Compounds</th>
<th>$R^2$</th>
<th>$\text{Repeatability}$</th>
<th>$\text{Intermediate precision}$</th>
<th>$I'$s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PEG</td>
<td>OV-1</td>
<td>IL-60</td>
<td>IL-60i</td>
</tr>
<tr>
<td>1</td>
<td>(Z,Z)-farnesol</td>
<td>0.998</td>
<td>0.999</td>
<td>0.998</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>(E,Z)-farnesol</td>
<td>0.997</td>
<td>Co-el</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>3</td>
<td>(Z,E)-farnesol</td>
<td>0.998</td>
<td>Co-el</td>
<td>0.998</td>
<td>1.000</td>
</tr>
<tr>
<td>4</td>
<td>(E,E)-farnesol</td>
<td>0.998</td>
<td>0.999</td>
<td>0.999</td>
<td>1.000</td>
</tr>
<tr>
<td>5</td>
<td>(Z)-α santalol</td>
<td>0.999</td>
<td>0.998</td>
<td>0.999</td>
<td>1.000</td>
</tr>
<tr>
<td>6</td>
<td>(Z)-α-trans-bergamotol</td>
<td>1.000</td>
<td>0.993</td>
<td>0.999</td>
<td>0.993</td>
</tr>
<tr>
<td>7</td>
<td>epi-β santalol</td>
<td>0.999</td>
<td>0.996</td>
<td>0.999</td>
<td>0.996</td>
</tr>
<tr>
<td>8</td>
<td>(Z)-β santalol</td>
<td>0.999</td>
<td>0.997</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Note: $R^2$, $\text{RSD} \% (0.5mg/ml)$, and Intermediate precision $\text{RSD} \% (0.5mg/ml)$ are listed for each column, along with the $I'$s values.
Figure 1

OV1701

IL60

IL60i

IL76

IL76i

IL111

IL111i
Figure 2

OV-1701

IL60

IL60i
Figure 3

Adsorption allergens

Normalized area
Figure 4

(a) IL60

(b) IL60i

c) IL60

d) IL60i

e) IL60

f) IL60i

g) IL60

h) IL60i
Figure 6

- **a) OV1**
  - UV (x100,000)
  - Min values: 34.25 to 36.75
  - Picoograms: 0.0 to 1.5

- **b) PEG-20M**
  - UV (x10,000)
  - Min values: 45.0 to 49.5
  - Picoograms: 0.0 to 3.5

- **c) IL60**
  - UV (x1,000)
  - Min values: 31.0 to 38.0
  - Picoograms: 0.0 to 3.0

- **d) IL60i**
  - UV (x100,000)
  - Min values: 37.0 to 40.0
  - Picoograms: 0.5 to 4.5

- **e) IL60i**
  - Optimized method
  - UV (x100,000)
  - Min values: 18.75 to 21.50
  - Picoograms: 0.5 to 4.0
Figure 7

(a) Adsorption of santalols and farnesols

(b) Tailing factor of santalols and farnesols