Ionic liquids as gas chromatographic stationary phases: how can they change food and natural product analyses?

Ionic liquids as gas chromatographic stationary phases: how can they change food and natural-product analyses?

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Abstract

The volatile fraction of natural products often consists of complex mixtures of isomeric and/or homologous components with similar structural and physical characteristics (e.g., mono- and sesquiterpenoids) that are not easy to separate simultaneously with conventional GC stationary phases, even when used with multidimensional systems. The introduction of ionic liquids (ILs) as stationary phases has opened up new perspectives in this field as their unique solvation properties result in uncommon selectivity, which is completely different to that of classic polydimethylsiloxane- (PDMS) and polyethyleneglycol- (PEG) based columns. Because of their peculiar selectivity and high inertness, IL-based columns have already found several applications in the natural-product field in mono- and multidimensional GC and preparative GC, leading to the exhaustive analysis of complex samples (including aqueous solutions), and the separation of challenging pair(s) of compounds. This article provides an overview of how IL-based columns can be exploited in the fields of food and natural products, explores the wide range of applications that have already been developed and highlights the main features of these promising stationary phases, which are expected to be further extended in the near future in particular for routine use.

Keywords

Ionic liquids; GC stationary phases; natural products; essential oils

Introduction

Natural-product analyses must cover a wide range of topics, from quality control to marker identification and the quantitation or isolation of diagnostic fractions for the elucidation of the structure of unknown markers. Gas chromatography (GC) is the technique of choice for the analysis of natural products that consist of volatile or semi-volatile components. These samples are, in general, complex mixtures that are often made up of isomeric and/or homologous components with similar structural and physical characteristics (e.g., mono- and sesquiterpenoids) that are not easy to separate simultaneously with conventional GC stationary phases (SPs), even when used with multidimensional systems. A further challenge with isomeric compounds is the fact that their mass spectra can be very similar, making chromatographic data (retention indices on different stationary phases), and thereby their unequivocal separation, indispensable for correct identification. They therefore require chromatographic systems in which efficiency and selectivity are properly combined to provide separations of as many diagnostic components (markers) as possible in a single run.

Column and GC technology have principally improved over recent decades in terms of the efficiency of the chromatographic system, at the detriment of selectivity, even in applications where column efficiency was higher than necessary, but where critical pairs were not separated because of a lack of selectivity [1]. The peculiar composition of natural products implies an in-depth re-consideration of the fundamental role played by the selectivity of a chromatographic system in separation.

In this context, the introduction of ionic liquids (ILs) as stationary phases for GC have opened up new perspectives thanks to their exceptional selectivity, which is completely different from that of classic SPs that are based on polydimethylsiloxane (PDMS) and polyethyleneglycol (PEG).

Ionic liquids are organic salts that possess melting points at or below 100°C. In most cases they are composed of an organic cation and an organic or inorganic anion, and are characterised by high thermal stability, low vapour pressure, and varying viscosity, conductivity and miscibility in different solvents [2]. The chemical structure of ILs (type of functional groups and/or anion/cation combination) can be tailored to allow them to undergo various solvation interactions. Their unique properties mean that they have found many applications in analytical chemistry, from sample preparation to GC and HPLC, being used as sorbent coatings and SPs, among other uses. In particular, as GC SPs, they impart unique and tunable selectivities toward a wide range of analytes with different functional groups, while maintaining chromatographic performance at levels that are comparable to those of classical GC stationary phases. IL-based GC columns are generally more polar than conventional columns, while providing similar or even higher maximum allowable operating temperatures (MOATs) than most PEG-based SPs. However, their solvation properties are different

to those of PDMS and PEG, as clearly demonstrated in some commercially available columns by Rodriguez-Sanchez *et al.*, [3], that are illustrated in Figure 1.

The popularity of IL-coated GC columns has increased after their commercial introduction by Supelco in 2008 and, to date, a satisfactory number of IL-coated columns with different characteristics are commercially available, although new derivatives are still under constant investigation as their full potential has not yet been fully explored.

Several excellent reviews have been published thus far on the developments, chromatographic properties and applications in the specific field of IL used as GC SPs; one of the most recent is by Trujillo-Rodríguez *et al.* [2]. This article is intended to give an overview of how IL-based columns can be exploited in a wide range of applications in the field of food and natural products. Although one of the main field of applications of these stationary phases is the analysis of the fatty acid methyl esters (FAMEs), this topic is not included in the present manuscript because it would require an extensive discussion and it was recently reviewed by Fanali *et al.* in 2017 [4].

Exploiting the peculiar selectivity of ionic liquids for the analysis of natural products

Most ILs that have been adopted as GC stationary phases mainly consist of one or more organic cations (mono or poly-cationic ILs) that contain nitrogen or phosphorus (usually phosphonium, imidazolium, pyridinium, or pyrrolidinium) in combination with one or more inorganic or organic anions. Due to their solvation properties, they are characterised by a retention mechanism of dual-nature; partition and interfacial adsorption. This property makes them able to separate both nonpolar and polar compounds and therefore extend the range of analyte polarity that they can separate compared to other well-established SPs. As mentioned above, samples in the plant field (for instance essential oils (EOs) or plant volatile fractions) are complex matrices consisting of compounds that are often homologous and/or isomers that belong to different chemical classes (mainly mono- or sesquiterpenoids), with a wide range of physicochemical properties. They include hydrocarbons and oxygenated compounds (aromatics, alcohols, aldehydes, ketones, esters, lactones, ethers, oxides, etc.) and others. The peculiar selectivity of ILs therefore makes these SPs of high interest in this field.

The first application of IL-based columns in this field was reported in 2007 by *Qi et al.*, [5]. They evaluated a geminal dicationic IL (1,9-di(3-vinylimidazolium)nonane bis[(trifluoromethyl) sulfonyl]imidate), in both its pure form and in a mixture with monocationic ILs and a polysiloxane diluent, in the analysis of fennel, cinnamon and nutmeg EOs. The authors compared the performance of the investigated columns to those of two conventional columns (HP-5 MS and HP-INNOWax) and

observed that the IL column, in a mixture with polysiloxane, possesses broader selectivity than those coated with either the pure IL or commercial polar and nonpolar SPs. It behaves as a fairly polar stationary phase in terms of selectivity and elution order and, at the same time, shows different selectivity to PEG as well as providing a higher number of separated compounds than all the other tested SPs. In 2009, the same group introduced a new generation of trigonal tricationic ILs that are based on four core structures ((A) mesitylene, (B) benzene, (C) triethylamine and (D) tri(2-hexanamido)ethylamine cores) that are attached to three identical imidazolium or phosphonium cationic moieties and paired with bis[(trifluoromethyl)sulfonyl]imide [NTf_2^-] [6]. They tested the new derivatives on the Grob mixture, a flavor and fragrance test sample consisting of a standard alcohol/alkane mixture, and FAME isomer separations. They found that the D series was very interesting because it not only gave the best separation for all test mixtures, but also eliminated peak tailing for alcohols and other H-bonding analytes, making them very promising in the field of natural products.

The popularity of IL-based stationary phases increased when they became commercially available. A set of commercial IL-based columns was introduced by Supelco in 2008. These include: SLB-IL59 and SLB-IL60, coated with 1,12-di-(tripropylphosphonium) dodecane paired with bis[(trifluoromethyl)sulfonyl]imide [NTf_2^-]); SLB-IL61 (1,12-di(tripropylphosphonium) dodecane paired with [NTf_2^-] and trifluoromethyl sulfonate); SLB-IL76 (tri-(tripropylphosphonium-hexanamido)-triethylamine 2[NTf_2^-]); SLB-IL82 (1,12-di(2,3-dimethyl-imidazolium) dodecane 2[NTf_2^-]); SLB-IL100 poly[1,9-di(3-vinylimidazolium)-nonane] 2[NTf_2^-], and; SLB-IL111 (1,5-di (2,3-dimethyllimidazolium) pentane 2[NTf_2^-]). The number in the column label indicates their polarity number (PN), i.e., the sum of the first five McReynolds constants, normalised to SLB-IL100 (set at P = 100).

The first application of a commercial IL column in the field of natural products was reported by Ragonese *et al.*, in 2011 [7]. These authors systematically evaluated the SLB-IL59 column, in terms of polarity, efficiency and selectivity, in the analysis of standard mixtures of typical EO constituents and in the analysis of lemon EO, comparing the results with apolar (SE52) and medium polar (PEG) columns. They evaluated the IL-based column, in terms of selectivity, stability-of-retention indices and quantification performance, and showed that the investigated SP has a polarity number that is comparable to that of PEG, but it exhibits higher thermal stability and different selectivity. Moreover, SLB-IL59 shows excellent stability-of-linear-retention indices (comparable to those of apolar columns) and better quantification performance than SPs of similar polarity.

Applications of the commercial IL-based columns in the food field have been reported on by Garcia-Pinto *et al.*, [8] and by Amaral *et al.*, [9]. In the first study, microextraction by packed sorbents (MEPS) was coupled with gas chromatography with a set of IL SPs (SLB-IL59, SLB-IL61, SLB-IL76 and SLB-IL100) to determine haloanisoles (responsible for the so-called cork off-flavour) in wines. On the other hand, Amaral *et al.*, have investigated the separation performance of SLB-IL60, SLB-IL76 and SLB-IL100 on coffee volatiles (extracted by head-space solid-phase microextraction, HS-SPME), and compared the results to those of a PEG column. SLB-IL60 showed comparable resolution and efficiency to that of the conventional column, but is able to identify compounds that are not baseline separated by PEG-based SPs, including 3,4-dimethyl-2,5-furandione, which is seldom reported in coffee literature.

In 2012, Cagliero *et al.*, [10] systematically evaluated the performance of all commercially-available IL-based columns (SLB-IL59, SLB-IL60, SLB-IL61, SLB-IL76, SLB-IL82, SLB-IL100, and SLB-IL111), in view of their routine application in flavour, fragrance and EO fields. Results were compared to those achieved using three conventional SPs (SE-52, OV-1701 and PEG–20 M). They analysed a standard mixture of suspected allergens and two EOs (cornmint and vetiver). The study concluded that the investigated columns could be used for a wide range of applications in these fields, mainly because of their non-common selectivity, in particular when functional-group based separation is required. However, they highlighted that further effort should be invested in column manufacturing to reduce their activity towards polar or active analytes in particular, in order to achieve inertness that is comparable to that of conventional columns, which is mandatory for quantitative analyses.

In response to this need for inertness, Supelco introduced, in 2016, a new series of highly inert IL-based columns, in which they modified the surface-treatment technology for three of their most popular IL SPs (SLB-IL60, SLB-IL76 and SLB-IL111). In 2017, Cagliero *et al.*, tested the newly introduced columns (SLB-IL60i, SLB-IL76i and SLB-IL111i), and compared their performance , including for quantitation, to those of the first generation of IL-based columns and to conventional SPs [11]. They observed that the new generation of IL-based inert columns were, in all aspects, competitive with the SPs of well-established use in the field (e.g., SE-52, OV-1, OV-1701 and PEG–20 M). In particular, they combine the selectivity of ionic liquids with high efficiency and inertness, since they produce highly symmetrical peaks, with areas that are independent of analyte structure and functionality. They also showed that inert IL columns provide good repeatability and intermediate precision, while also enabling percentage normalisation and true quantitation methods to be used.

One of the main features of ILs is that their chemical structure can be tailored to produce different solvation interactions and therefore provide unique and task-specific chromatographic selectivities. In this respect, Mazzuccotelli *et al.*, have performed an in-depth investigation of the use of two phosphonium-cation based ILs; trihexyl(tetradecyl)phosphonium, [P_{66614}^{+}]) in combination with two different anions, bis[(trifluoromethyl)sulfonyl]imide [NTf_2^{-}] and chloride [Cl^{-}] [12]. They evaluated the chromatographic performance and selectivity of the two stationary phases in a model mixture of compounds that are typical of the flavour, fragrance and EO fields; a mixture of 29 volatile allergens and two EOs of different complexity (sage and vetiver EOs). The columns that were coated with the investigated IL SPs showed high efficiency and inertness. Furthermore, the two phosphonium based ILs exhibited completely different selectivity, and [P_{66614}^{+}] [Cl^{-}] was characterised by a high hydrogen-bond basicity interaction [13], that resulted in high retention, and selectivity that was based on the analyte functional groups. [P_{66614}^{+}] [NTf_2^{-}] is an IL in which none of the Abraham model coefficients prevails significantly over the others [13], and is characterised by shorter retention, and selectivity that is mainly related to analyte volatility and polarity. This different and tunable selectivity is evident from the GC–MS patterns of sage EO reported in figure 2.

Exploiting the orthogonality of ionic liquids for multidimensional GC applications

The complexity and diversity of the metabolic composition of natural products in some cases exceed the peak capacity that can be achieved with one-dimension GC (1D-GC). Multidimensional-GC (MDGC), i.e., comprehensive two-dimensional GC (GC×GC), heart-cut GC and higher order GC designs, is required for an exhaustive investigation of the major and minor components of these samples, in particular in the –omics field. The set-up of MDGC entails the simultaneous use of different columns coated with orthogonal SPs, i.e., those with different selectivity and polarity. In this respect, IL-based stationary phases are an excellent alternative to the usual PEG columns. Furthermore, Novalchai *et al.* demonstrated that, because of their peculiar temperature-dependent linear solvation energy relation-ship (LSER), a tunable separation pattern can be achieved with the same IL-based SP and by simply varying the ^{1}D column dimension [14].

The first application of an IL-based column for a GC×GC separation in the field of natural products was reported by Purcaro *et al.*, in 2010 [15]. The study was focused on the applicability of high-speed quadrupole MS to quantify 24 allergens and a perfume sample. Supelco SLB-IL59 was used as a second dimension because of its polarity, which is comparable to PEG, and its higher thermal stability and lower bleeding.

Tranchida *et al.,* used a SLB-IL60 column, with a polarity that is similar to that of SLB-IL-59, as a second dimension in GC×GC to evaluate the potential of high-speed triple quadrupole mass spectrometer, operating in different acquisition modes (scan/MRM, MRM, scan/SIM and SIM), in the untargeted and targeted analyses of a contaminated mandarin EO, and for the detection and quantification of five pesticides in a spearmint EO [16].

Chin *et al.,* have proposed the use of SLB-IL59 as the first dimension in an integrated comprehensive/multidimensional GC system, coupled with MS and olfactometry, for the aroma analysis of coffee [17]. The authors observed that the use of this IL column was beneficial, compared to the use of a FFAP column, because: i) most compounds elute after 10 minutes, improving cryogenic modulation, and; ii) the later elution of coffee volatiles leads to a higher elution temperature, and, thereby, shorter ^{2}D retention times, which limits the possibility of analyte wrap-around. The adoption of an IL-based stationary phase in the first dimension for this GC×GC separation resulted in better occupation of the 2D space.

For the same reason, Wong *et al.,* applied an analogous column set up (with SLB-IL59 as first dimension) for the qualitative analysis of sesquiterpenes and diterpenic acids in a *Copaifera multijuga* Hayne oleoresin using GC×GC and GC with conventional and cold-electron ionisation MS [18].

The same group used an IL-based column in the second dimension for the authentication of Australian tea tree EOs using enantioselective-GC×GC-ToF-MS analysis. A column set-up, consisting of 2,3-diethyl-6-tertbutyldimethylsili-β-cyclodextrin as chiral selector in ^{1}D and SLB-IL61 in ^{2}D, was applied to avoid the coelutions that were observed using a PEG column in ^{2}D. The column set-up with the IL-based column in the second dimension enabled the enantiomeric composition of the three chiral markers (limonene, terpinen-4-ol, α-terpinolene) of authentic Australian tea tree EOs to be effectively evaluated and possible adulterations with samples of different origin to be determined in commercial products [19].

Sciarrone *et al.,* have applied heart-cut MDGC with a low bleed IL-based secondary column in combination with combustion-isotope ratio mass spectrometry (IRMS) and simultaneous quadrupole MS detection to authenticate truffles (*Tuber magnatum* Pico), and food that contains truffles, by monitoring the δ^{13}C of its odorous key aroma compound (bis(methylthio)methane) [20]. SLB-IL59 was used as the second dimension of the MDGC system to minimise the effect of column bleeding on a δ^{13}C measurement that was too high with a PEG column.

Finally, Yan *et al.,* have very recently introduced a novel platform, for the high-resolution characterisation of multicomponent samples, that consists of sequential hybrid three-dimensional gas

chromatography with accurate mass spectrometry (3D GC–accTOFMS), in which a first non-polar column is on-line and combined, through microfluidic heart-cutting, with a GC×GC system using a PEG column as the second dimension, and an ionic liquid column (SLB-IL59) as the 3rd dimension (GC_{np}−GC_{PEG}×GC_{IL}) [21]. The system was used to analyse oxygenated sesquiterpenes in hop (*Humulus lupulus* L.) EO and agarwood (*Aquilaria malaccensis*) oleoresin. For these samples, it was proven that MDGC, with an apolar/PEG column set-up, was not sufficient to provide the separation of all the components in the matrices, therefore making a third SP, with complementary selectivity (the IL column), necessary. Figure 3 reports the sequential GC_{np}−GC_{PEG}×GC_{IL}−accTOFMS analysis of the sesquiterpenoids in an *A. malaccensis* oleoresin sample.

Ionic liquids and preparative GC for the isolation of challenging compounds

In the field of natural products, the isolation of pure compounds from a complex matrix is fundamental both for the identification (correct structural elucidation of new compounds), and/or isolation of isomers/enantiomers that are biosynthesised by plants, but that are difficult to obtain via organic synthesis. Column selection is of crucial importance in preparative GC (prep GC). On the one hand, higher amounts of samples are usually injected (thus requiring high capacity systems), while, on the other, it is important to isolate the target compounds from interfering/coeluting molecules in the chromatogram. This need makes the adoption of highly selective SPs mandatory. On the basis of these considerations, Mondello's group have developed a novel prep GC system that is based on heart-cut MDGC and is equipped with Deans-switch devices and makes use of IL columns combined with others that are coated with more conventional polar and apolar SPs.

The first article reporting the use of this system was published in 2012 by Sciarrone *et al.*, [22]. They adopted a three-dimension Deans-switch multidimensional preparative gas chromatographic system to isolate carotol from a carrot seed EO. They optimised the injection conditions, column set-up and collection conditions for the effective isolation of the compound from the complex mixture. They found that the best combination was the set-up consisting of 5% diphenyl-polyethylene glycol-ionic liquid SPs (SLB-IL59), because of their different selectivities and high separation-power capabilities. They adopted wide-bore columns (0.53 mm d_f) that were coated with thick films of SPs (5, 2, 0.85 μm for the first, second and third dimension, respectively). Figure 4a-c reports the combination of the profiles of the carrot-seed oil in stand-by and heart-cut conditions of the three dimensions. The purity of the carotol isolated using the developed system was of around 99.6% (Figure 4d). The same group adopted this approach in a subsequent study that aimed to isolate an unknown component of wampee EO (derived from *Clausena lansium* Skeels leaves). The isolated compound was then identified as

(2E,6E)-2-methyl-6-(4-methylcyclohex-3-enylidene)hept-2-enal using nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS) [23]. Also in this case, the authors highlighted the need for a high-resolution GC step (and therefore, for SPs with different selectivity) before analyte collection.

Further evolutions in the prep-MDGC system were later introduced: Sciarrone *et al.*, proposed, in 2014, a combination of the prep GC-GC-GC system with an online LC pre-separation step, operated in normal phase [24]. The system was tested as a means to isolate two trace components (α-amorphene and β-vetivone) from the, very complex, vetiver EO. The IL-based SP SLB-IL59 was used as the second-dimension column. Lastly, Sciarrone *et al.*, implemented, in 2016, the GC-GC-GC system which boasts of the ability to collect samples after each chromatographic dimension. The developed system was then applied to the isolation of components from patchouli EO [25]. They showed that patchouli alcohol could be collected after the first column (SE-52), α-bulnesene after a second column, which was coated with PEG, whereas a SLB-IL60 column in the third dimension was necessary to isolate α-guaiene.

Water-compatible ionic liquids for the analysis of water and aqueous samples

Analyses in the food and natural-products field often involve the determination of water content or the analysis of products that are formulated or diluted in aqueous media. The direct injection and analysis of these samples using capillary columns that are coated with the most common stationary phases is often discouraged as they are poorly compatible with water, resulting in possible phase degradation and limited column performance. Water analyses and the direct injection of aqueous samples (DAI) are often carried out in packed columns, although they too have some drawbacks, such as broad tailing peaks, peak overlap and irreproducibility [26].

Ionic liquids (ILs) have also shown great potential in this context thanks to: i) their water and oxygen stability, and; ii) the fact that derivatives that not only have good selectivity but also produce water symmetrical peaks can be designed.

In 2011, Armstrong's group introduced IL-derivatives that are based on phosphonium and imidazolium-PEG cations in combination with anions consisting of 2 or 3 units of trifluoromethanesulphonate. These derivatives produce a narrow and symmetrical water peak, which results in improved peak-area reproducibility and in its beneficial separation from other solvents and volatile substances [26].

The same group used GC with columns that were coated with these derivatives, together with Thermal Conductivity Detection (TCD) and/or Barrier Discharge Ionisation (BID) Detection, to quantify the water/ethanol content in various commercial products, including beverages [27]. An analogous approach was applied by Frink *et al.,* to determine the water content in honey [28]; static head space (HS)-GC was coupled with TCD and BID, used as detectors, together with the PEG-linked geminal dicationic ionic-liquid column.

In 2016, Supelco made a series of water-compatible IL columns, which possess a variety of retention properties and are based on the above-mentioned ILs, commercially available under the trade name Watercol™.

In 2018, Cagliero *et al.,* reported the results of a study that extended the use of commercially-available water-compatible IL columns to the direct analysis of aqueous samples in the fragrance and EO fields, which was carried out using GC with thermal conductivity (TCD) and/or flame ionisation detectors (FID) [29]. The columns investigated were Watercol™ 1460, which is coated with tri-(tripropyl-phosphoniumhexanamido)-triethylamine trifluoromethanesulfonate, and Watercol™ 1910, which is coated with 1,11-di-(3-hydroxyethylimidazolium)-3,6,9-trioxaundecane trifluoromethane sulfonate. They evaluated column performance, in terms of stability, inertness, efficiency and selectivity, on a mixture of 29 compounds in the perfume field, 24 of which are included in the list of EU-suspected allergens, that were diluted in a hydroalcoholic solvent. They also tested the qualitative and quantitative performance of the investigated SPs on peppermint, lavender and tea tree EOs that were diluted in aqueous media, and commercial perfumes. They showed that water-compatible IL-based stationary phases can be successfully used for the qualitative and quantitative analysis of aqueous samples, thus avoiding the time-consuming and sometimes discriminating sampling procedures that are needed to extract fractions or analytes of interest with solvents that are suitable for use with conventional columns.

More recently, Sgorbini *et al.,* [30] have successfully used Watercol™ columns in a study that compared different methods to measure transfer rate and human intake of volatile bioactive compounds from herbal teas prepared with medicinal and aromatic plants. They compared the direct aqueous injection (DAI) of the herbal teas using the water-compatible IL-based columns and GC-FID with DI-SPME, in combination with the GC-MS of the same samples. The two methods gave comparable results with high repeatability, linearity and accuracy. The DAI-GC-FID method reduced the total analysis time by a factor of two and did not suffer from the potential discrimination of analytes, although it was found to be less sensitive than conventional methods that involve sample preparation, as it does not entail an analyte concentration step. The authors therefore concluded that

the two methods were fully complementary and their adoption depends on the amount of marker(s) to be quantified.

In both studies on the analysis of aqueous samples using water-compatible IL-based SPs in the field of natural products, the authors emphasised that, with a view to making their use in quality control routine, mass spectrometry should be used as the GC detector in order to make their use fully compatible with regulatory requirements. Mazzucotelli *et al.*, have very recently applied narrow-bore water-compatible IL-based columns to the direct quantitative analysis of aqueous samples with GC-MS in the perfume field [31]. After a careful tuning of column dimensions, and injection and MS conditions, the study showed that aqueous samples could be systematically and routinely analysed by direct injection-GC-MS with water-compatible narrow bore IL columns. The chromatographic performance of both investigated Watercol^{TM} (1460 and 1910) were consistent over time and in-line with that of the corresponding narrow-bore columns coated with conventional SPs.

Outlook

Their unique solvation properties grant ILs peculiar selectivity, which is not comparable to those of PDMS and PEG columns, when they are used as GC SPs. This makes them very useful in the food and plant fields, where selectivity plays a fundamental role, because of the complexity of the samples and the similarity of their components. At the same time, the optimisation of column preparation that has been carried out in recent years has driven a drastic improvement in chromatographic performance (in terms of efficiency and inertness), meaning that high performance columns for qualitative and quantitative analyses are now available. IL-based columns have already found several applications in the field of natural products in mono- and multidimensional GC and preparative GC, and are now performing the exhaustive analysis of complex samples (including aqueous solutions), and the separation of challenging compounds. The large number of applications of IL-based SPs shows that they are now ready for inclusion in the columns set that is routinely used in quality-control laboratories. The number of specific applications of this technique is expected to increase significantly, in particular in the analyses of complex and challenging samples. However, although the stability of the chromatographic performances (and therefore of retention indices) of these columns has been extensively proved, very few linear retention indices are reported in the literature. A library of indices for compounds in these fields measured with the most common IL-based stationary phases is therefore desirable being this parameter essential for their correct identification.

Furthermore, the full potential of using ILs as GC stationary phases has only partially been investigated. The interchange of anions and cations results in an almost unlimited number of chemical combinations and, thereby, of chemical characteristics and chromatographic properties that have only been partly explored at present. The study on new IL derivatives must therefore be continued, while the main limits of some of these compounds, including their relatively high vapour pressure limiting maximum allowable operating temperature (MAOT), must also be taken into consideration. In this context, very promising perspectives have been opened up by the introduction of perarylated sulphonium and phosphonium IL-based stationary phases that have shown extremely high thermal stability and MAOTs of up to 350°C [32]. Further advances are therefore expected to expand their applications in the food and natural product analysis to satisfy the ever increasing demand of exhaustive and diagnostic patterns for complex samples in particular for fields such as nutrimetabolomics, foodomics and plant volatilomics.

Conflict of interest

The authors declare no conflict of interest

Figures

Figure 1: three-dimensional PCA scores plot obtained on the Abraham coefficient parameters calculated at 120°C for 45 non-ionic capillary columns together with the commercial IL-based columns. Dashed lines indicate the trend of distribution of siloxane-type SPs substituted with trifluoropropyl (1), phenyl (2) and cyanopropyl (3) groups. Clusters of poly(ethylene glycol) (cluster I) and IL (cluster II) phases are highlighted by circles. The figure is adapted with permission from Ref. [3]

Figure 2: GC–MS patterns of sage essential oil with $[P_{66614}^{+}]$ $[Cl^{-}]$ (a) and $[P_{66614}^{+}]$ $[NTf_2^{-}]$ (b) IOL-based columns. Temperature program: from 50°C to 200°C (5 min) at 10°C/min. Legend: Mon. Hydr.: monoterpenoids hydrocarbons, Sesq. Hydr.: sesquiterpenoids hydrocarbons, Ox. comp.: oxygenated compounds. The figure is adapted with permission from Ref. [15].

Figure 3: Sequential GC_{np}–GC_{PEG}×$GC_{IL(SLB\text{-}IL59)}$−accTOFMS analysis of the oxygenated sesquiterpenes in *A. malaccensis* oleoresin. (Ai) $^{1}D_{np}$ FID response, the region to be H/C is denoted by the dotted rectangle, with an inset (Aii) of the expansion of the target region 54–59 min. (Bi) Heart-cut MDGC–accTOFMS analysis of the target region 54–59 min with an inset (Bii) of the $^{1}D_{np}$ FID response showing 54–59 min H/C to $^{2}D_{PEG}$. (C) GC_{np} −GC_{PEG}×GC_{IL}−accTOFMS analysis of the target region 54–59 min; red dots are autogenerated by the software for the detected peaks. The figure is adapted with permission from Ref. [15]. For the numbering of the peaks in (Bi) and (C) see Ref. [15].

Figure 4: Stand-by (black traces) and heart-cut (pink traces) chromatograms, relative to the separation of the carrot seed oil in the first-A (5%phenyl-SP), second-B (PEG SP), and third-C (SLB-IL59) GC dimension. D: GC-FID chromatogram of carotol, collected by means of the prep MDGC instrument. The figure is adapted with permission from Ref. [24].

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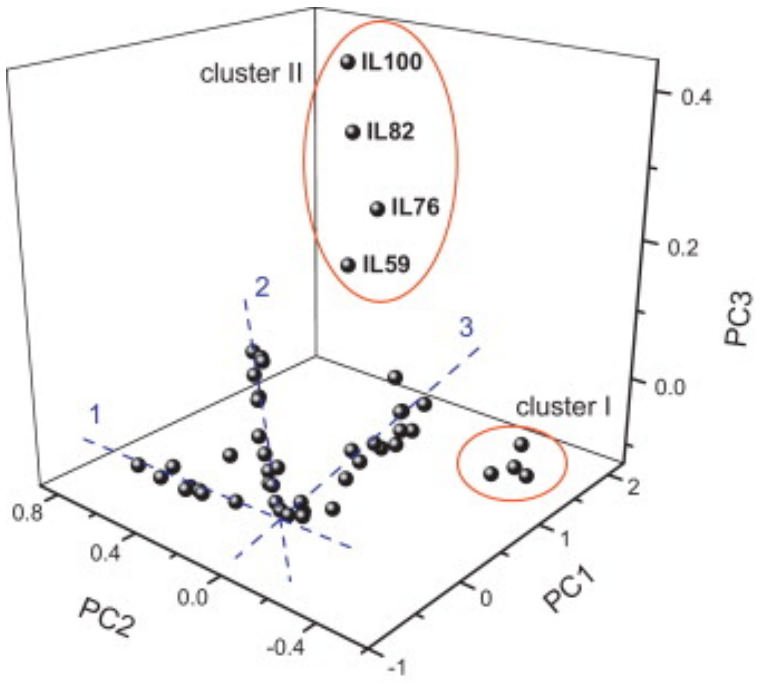
Figure 1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IL82
IL76
IL59
cluster I
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2
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PC1
PC2
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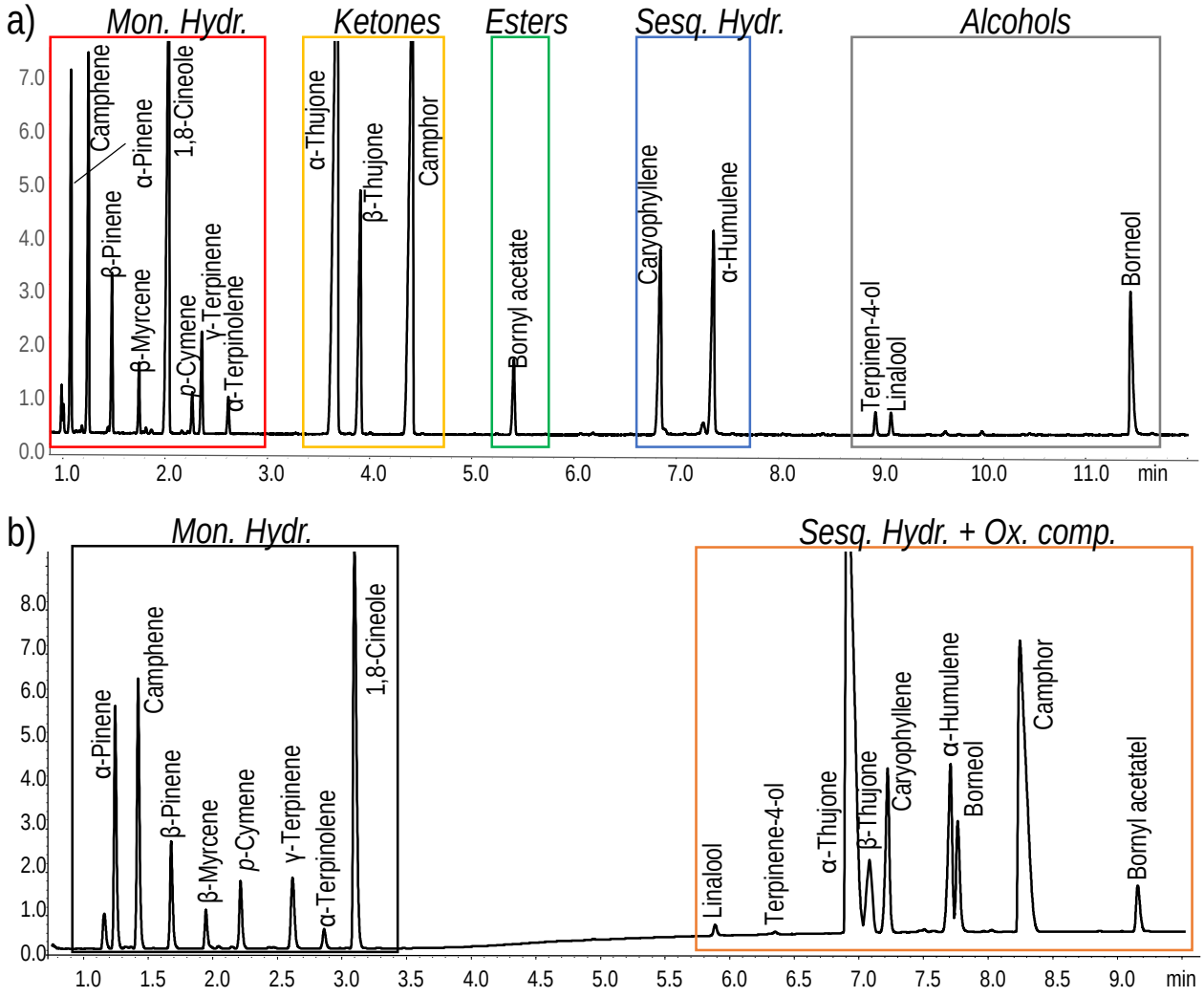
0.2
0.4

Figure 2


a)
Mon. Hydr.
Ketones
Esters
Sesq. Hydr.
Alcohols
Camphene
α-Pinene
1,8-Cineole
β-Pinene
β-Myrcene
p-Cymene
γ-Terpinene
α-Terpinolene
α-Thujone
β-Thujone
Camphor
Bornyl acetate
Caryophyllene
α-Humulene
Terpinen-4-ol
Linalool
Borneol
min
b)
Mon. Hydr.
Sesq. Hydr. + Ox. comp.
α-Pinene
Camphene
β-Pinene
β-Myrcene
p-Cymene
γ-Terpinene
α-Terpinolene
1,8-Cineole
Linalool
Terpinene-4-ol
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β-Thujone
Caryophyllene
α-Humulene
Borneol
Camphor
Bornyl acetatel
min

Figure 3

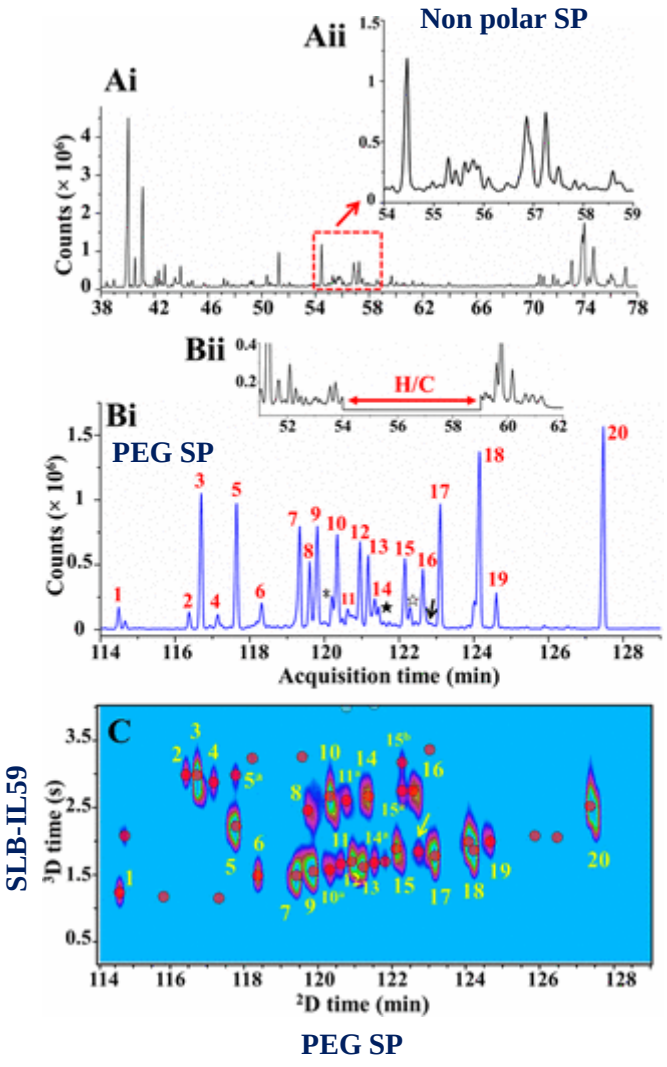
Ai
Aii
Non polar SP
Counts (× 10⁶)
Bi
Bii
PEG SP
H/C
Acquisition time (min)
C
SLB-IL59
³D time (s)
²D time (min)
PEG SP

Figure 4