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**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1660461> since 2019-01-07T17:48:47Z

*Published version:*

DOI:10.1007/s00216-018-0922-0

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**This is the author's final version of the contribution published as:**

[C. Cagliero, C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils, *Anal Bioanal Chem*, (2018)., <http://dx.doi.org/10.1007/s00216-018-0922-0>]

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# Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils.

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**Abstract** - Fragrances and products deriving from essential oils are often formulated or diluted in aqueous media, usually ethanol/water. Gas Chromatography (GC) is the technique of choice to analyze volatiles. However, when using columns coated with conventional stationary phases its application to aqueous samples often requires time-consuming and/or discriminative sample preparation techniques to extract the target analytes from the aqueous medium, so as to avoid its direct injection. In GC with conventional columns, water produces peak asymmetry, poor sensitivity and efficiency, strong adsorption, stationary phase degradation and, last but not least, it is not easy to detect reliably when present in high amounts. In 2012, Armstrong's group introduced new fully-water-compatible ionic-liquid (IL)-based GC capillary columns based on phosphonium and imidazolium derivative cations combined with trifluoromethanesulphonate. These columns were recently made available commercially by Supelco, under the trade name Watercol™. These derivatives maintain IL's unique selectivity and chromatographic properties, and enable water to be used as injection solvent, thus avoiding the sample preparation procedures required by conventional columns. This study reports and critically discusses the results of commercially-available water-compatible IL columns for direct analysis of aqueous samples in the fragrance and essential oil fields by GC with thermal conductivity (TCD) and/or Flame ionization detectors (FID). The results showed that water-compatible IL-based stationary phases can successfully be adopted for qualitative and quantitative analysis of fragrances and essential oils directly diluted in aqueous solvents. On the other hand, the study also shows that their inertness needs to be further increased and (possibly) the range of operative temperature extended when water is the main solvent of the sample.

**Keywords** - Ionic Liquids; GC; Aqueous samples; Water-compatible stationary phases; Essential oils; Fragrances

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## INTRODUCTION

Gas Chromatography (GC) is the technique of choice for the analysis of fragrances and essential oils. However, these products are often formulated or diluted in aqueous media, usually ethanol/water in different ratios. Quali-quantitative analysis of their composition, or of one or more specific components, either as markers of quality or that are limited by regulatory authorities (e.g. suspected allergens in perfumes or cosmetics by the EU [1, 2]) is often required. When they are in an aqueous medium, this often entails the adoption of sample preparation techniques to extract target analytes that can operate a discrimination of the components of the sample, or the dilution with compatible solvents that affects sensitivity to avoid direct injection in GC of aqueous solutions. A similar approach is also necessary when water is an analyte to be quantified, since with columns coated with conventional stationary phases it produces degradation of those phases, peak asymmetry, poor sensitivity, poor efficiency, and strong adsorption; further, it cannot be detected in high amounts, since it produces broad peaks with low peak area repeatability, and unsatisfactory limits of detection and quantitation [3]. In the past, these problems were solved, although not satisfactorily, by using packed columns filled or wall-coated (PoraPLOT) with molecular sieves as stationary phases.

Over the last two decades, ionic liquids (ILs) have been shown to have great potential as GC stationary phases [4]. In 2012, Armstrong *et al.* showed that some ionic-liquid (IL)-based GC capillary columns have not only good selectivity but also high stability and compatibility toward water as analyte, compared to traditional commercial columns [3]. Moreover, these stationary phases provide good peak symmetry, thus avoiding chromatographic interference with other analytes. One of IL's main advantages is that their chemical structures can be custom-designed to add compatibility with specific compounds to their selectivity. The original ILs proposed by Armstrong's group were based on phosphonium and imidazolium derivative cations, combined with anions consisting of 2 or 3 units of trifluoromethanesulphonate; the group proposed a number of applications to test the reliability of the above and other ILs derivatives, as GC stationary phases to measure the water content of matrices in different fields, including in active pharmaceutical ingredients [5, 6] and in honey [7], and also to measure the water/ethanol content of various consumer products. More recently, Supelco introduced water-compatible IL columns commercially under the trade name Watercol™, with different retention properties based on the above mentioned ILs (for details see experimental [8]).

To the best of the authors' knowledge, the adoption of water compatible ILs as GC stationary phases when water is the main solvent, as is often the case in the fragrance and essential oil fields, has not yet been investigated. This study evaluates the quali-quantitative performance of water-compatible IL columns for

direct analysis of aqueous or water/ethanol samples in the fragrance and essential oil field by GC, with Thermal Conductivity (TCD) and/or Flame Ionization Detectors (FID). TCD was applied to detect the peak of water while FID was used to increase the response of target compounds with water as solvent.

## **MATERIALS AND METHODS**

**Samples.** A mixture of ethanol and water in a 1:1 ratio was analyzed to test the columns' performance when analyzing aqueous solutions. The Grob test mixture, [9] consisting of a mixture of decane, dodecane, 1-octanol, methyl decanoate, methyl undecanoate, methyl dodecanoate, 2,6-dimethylphenol, 2,6-dimethylaniline, dicyclohexylamine, and 2-ethylcaproic acid in hexane and methylene chloride, was purchased from Sigma–Aldrich (Milan, Italy) and analyzed as such. The suspected allergen standard mixture included 29 compounds: **1:** limonene (CAS: 138-86-3), **2:** linalool (CAS: 78-70-6), **3:** estragole (CAS: 140-67-0), **4:** phenylacetaldehyde (CAS: 122-78-1), **5:** methyl 2-octynoate (CAS: 111-12-6), **6:** citronellol (CAS: 106-22-9), **7:** geraniol (CAS: 106-24-1), **8:** benzyl alcohol (CAS: 100-24-1), **9:** neral (CAS: 106-26-3), **10:** geranial (CAS: 141-27-5), **11:**  $\alpha$ -isomethyl ionone (CAS: 15789-90-9), **12:** methyl eugenol (CAS: 93-15-2), **13:** hydroxycitronellal (CAS: 107-75-5), **14:**  $\alpha$ -ionone (CAS: 127-41-3), **15:** eugenol (CAS: 97-53-0), **16:** lilial (CAS: 80-54-6), **17:** cinnamaldehyde (CAS: 104-55-2), **18:** anisyl alcohol (CAS: 1331-81-3), **19:** farnesol isomers (CAS: 4602-84-0), **20:** cinnamyl alcohol (CAS:104-54-1), **21:** amyl cinnamaldehyde (CAS: 122-40-7), **22:** hexyl cinnamaldehyde (CAS: 39350-49-5), **23:**  $\alpha$ -pentylcinnamyl alcohol (CAS: 14316-49-5), **24:** vanillin (CAS: 121-33-5), **25:** lyral isomers (CAS: 130066-44-3), **26:** coumarin (CAS: 91-64-5), **27:** benzyl benzoate (CAS: 120-51-4), **28:** benzyl salicylate (CAS: 118-58-1), and **29:** benzyl cinnamate (CAS: 103-41-3). They were solubilized in a mixture of water and ethanol 1:1 at a concentration of 500 mg L<sup>-1</sup>. A stock quantitation standard mixture consisting of linalool (**2**), linalyl acetate (**30**) and  $\alpha$ -ionone (**14**) was prepared by adding the pure standards (purchased from Sigma–Aldrich) to an appropriate volume of ethanol at an initial concentration of 10 g L<sup>-1</sup>. Working solutions were prepared by diluting the stock solution in appropriate volumes of a mixture of water and ethanol 1:1. A set of five commercial perfumes was purchased from a local market and injected as such. 2-Methylbutanol (from Sigma–Aldrich) at a concentration of 1 g L<sup>-1</sup> as internal standard was added to both calibration solution and commercial perfumes.

The essential oils of peppermint (*Mentha x piperita* L.) and lavender (*Lavandula angustifolia* Mill.) were obtained by hydrodistillation following the European Pharmacopoeia [10], while tea-tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) essential oil was supplied by Erboristeria Magentina SrL (Poirino, TO, Italy). The essential oils were solubilized in a mixture of water and ethanol 1:1 at a concentration of 5

g L<sup>-1</sup> before analysis. Deionized water (18.2 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Merck, Milan, Italy) while ethanol (99.9%) was purchased from Sigma Aldrich (Milan, Italy).

**Instrumental set-up.** Analyses were carried out on a Shimadzu GC-FID-TCD system consisting of a Shimadzu GC 2010 equipped with FID in parallel with a TCD, the two detectors were alternately operated depending on the experiments; data were processed with GC Solution 2.53SU software (Shimadzu, Milan, Italy). Analyte identification was performed by GC-MS using a Shimadzu QP2010-PLUS GC-MS system equipped with Shimadzu GCMS Solution 2.51 software.

**Columns.** GC analyses were carried out with two 30 m × 0.25 mm d<sub>c</sub> × 0.20 μm d<sub>f</sub> Watercol™ columns of different polarities, i.e. Watercol™ 1460 (non-bonded Tri(triisopropylphosphoniumhexanamido)-trimethylamine trifluoromethanesulfonate) and Watercol™ 1910 (non-bonded 1,11-Di(3-hydroxyethylimidazolium)3,6,9-trioxaundecane trifluoromethanesulfonate). The results were compared with those from a 30 m × 0.25 mm d<sub>c</sub> × 0.25 μm d<sub>f</sub> column coated with 14%-cyanopropylphenyl 86%-dimethylpolysiloxane (OV-1701) and from two other commercial ionic liquid columns, namely SLB-IL60 and SLB-IL60i (30 m × 0.25 mm d<sub>c</sub> × 0.20 μm d<sub>f</sub>). All IL columns were from Supelco (Bellefonte, PA, USA) while OV-1701 column was from MEGA (Legnano, Mi, Italy).

**Analysis conditions.** Injector temperature: 220°C, liner volume: 1 mL. FID temperature: 230°C, FID sampling rate: 40ms, TCD temperature: 250°C, TCD sampling rate: 40ms, TCD makeup gas: He (flow: 1 ml min<sup>-1</sup>). All temperatures were reduced to 200°C for 1910. Temperature program for all columns (with the exception of Watercol™ 1910): i) 40°C//2°C/min//230°C(2min) for analyses of water:ethanol mixture, allergens, quantitation standard mixture and perfumes, ii) 40°C//3°C/min//230°C(2min) for Grob test, iii) 70°C//3°C/min//230°C(2min) for essential oils. The final temperature was set at 180°C for analyses with Watercol™ 1910, while the time of the final isotherm was increased to 30 minutes. Flow rate (He): 1 mL min<sup>-1</sup>. Injection mode: split. Split ratio: 1:100 for perfumes and quantitation standard mixture, 1:20 for Grob test, allergens and essential oils, while a split ratio of 1:5 was used for TCD analyses. The MS operated in electron impact ionization mode (EI) at 70 eV, scan rate: 666 u/s, mass range: 35–350 m/z.

**Analytes identification and quantitation.** Analytes were identified by comparing their mass spectra and linear retention indices (*I*<sub>T</sub><sup>s</sup>) to those of authentic standards, or to those in commercial or in-house libraries, or to literature data. Retention indices were calculated versus a C9-C30 hydrocarbon solution analyzed under the conditions reported above.

The external standard calibration method was applied to quantify the target components of the commercial perfumes with GC-FID, by building a calibration curve for each compound. The analytical performances of the quantitation methods were tested for analyte repeatability and intermediate

precision, and linearity  $R^2$ . Peak area repeatability was measured by analyzing each point of the calibration curves five times by GC-FID, while intermediate precision was determined by analyzing aliquots of the same samples every three weeks over a period of three months. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by injecting the quantitation standard mixture at increasing dilutions until reaching a signal:noise ratio of 3:1 (LOD) and 10:1 (LOQ).

**Head Space (HS) SPME sampling conditions.** A 1 cm PDMS/DVB OC (over coated) fiber from Supelco (Bellefonte, USA) was used. Sampling conditions: amount: 10  $\mu$ L, sampling temperature: 50°C, sampling time: 15 min.

## **RESULTS AND DISCUSSION**

This study evaluates the performances of the two water-compatible columns, i.e. Watercol™ 1460 (hereafter 1460) and Watercol™ 1910 (hereafter 1910). The numbers, 1460 and 1910, indicate the respective water Linear Retention Indices ( $I^T_s$ ) on the two columns, calculated versus a hydrocarbon mixture. The study tested these columns qualitatively in terms of stability, intermediate precision of linear retention indices ( $I^T_s$ ), peak width and symmetry (tailing factor), and analyte recovery, and quantitatively for their linearity, repeatability, LOD, and LOQ. Tests were carried out with a 1:1 ethanol/water mixture, the Grob test, a standard mixture of regulated suspected allergens included in the EU list [2], and on real-world samples consisting of ethanol/water solutions of three essential oils of interest in the cosmetic, food and pharmaceutical fields (lavender, peppermint and tea-tree oil) and five commercially available perfumes. All results were compared to those obtained with a conventional OV-1701 column and/or a commercial inert IL column (SLB-IL60i). In particular, SLB-IL60i was chosen because it had been shown to give comparable or sometimes better chromatographic results than those of columns coated with conventional stationary phases (polydimethyl siloxane or PEG20M) [11]. Lastly, where necessary the Watercol™ results were compared to those obtained for the same analytes dissolved in conventional solvents, or resulting from suitable sample preparation procedures.

### **Column performances and selectivity**

The performances of the 1460 and 1910 columns were evaluated in terms of stability to water injection, inertness and efficiency.

*Column performance after water injection* - The first series of experiments evaluated the stability of the columns investigated when water is injected as main solvent. The consistency of column performance when large amounts of water are injected was evaluated with a 1:1 water/ethanol solution. The sample



was injected in both 1460 and 1910 columns installed in a GC-TCD system (Figure 1), 20 times in the same day, and three consecutive times each day for the subsequent 10 days to measure water and ethanol peak areas, retention times, and linear retention indices ( $I^T_s$ ). Retention of water with the two columns significantly varied because of their widely differing selectivity, as well as their relative retention *versus* ethanol, which, conversely, was rather constant. In particular, with 1910, the  $I^T_s$  difference between water and ethanol was about 450 units (for analysis conditions see experimental). The results of 50 injections of the water/ethanol solution showed a very stable performance,  $I^T_s$  RSD% being below 2% for both analytes analyzed on both columns, and peak abundance was highly consistent, with RSD % on their absolute areas never exceeding 4%.

*Column performance with the Grob test* – Simultaneously, the Grob test was also injected for 10 consecutive days into the two investigated columns, to check their inertness and efficiency *versus* a set of model analytes. Table 1 reports retention times, peak width, tailing factors, and recovery of the Grob test components, compared to a conventional SLB-IL60i. The recovery percentage vs. SLB-IL60i was measured using 1-octanol as internal reference for normalization.

As expected, the two columns differed in terms of both retention and selectivity. Retention of 1910 was lower than that of 1460, the total analysis time for the components detected being around 20% less. Their selectivity *versus* the Grob test components differed quite widely, making them complementary in particular for the analysis of complex mixtures.

With 1460, most peaks were narrow and with a good symmetry, with the exception of dicyclohexylamine and 2-ethylhexanoic acid, whose peak shapes were significantly distorted, their tailing factors being 2.00 and 2.61 respectively. The 2,3-butandiol peak width was acceptable (0.060) but it was strongly adsorbed (about 90%), explaining its high tailing factor (1.847).

With 1910, under the recommended analysis conditions, dicyclohexylamine and 2-ethylhexanoic acid did not elute after 75 min, probably indicating an irreversible interaction with the stationary phase, and possibly because the maximum allowable operative temperature (MAOT) of this stationary phase is 180°C [8]. All other peaks eluted with good symmetry, with the exception of methyl dodecanoate (and to a lesser extent of methyl undecanoate). Their low tailing factor is due to peak-leading, probably related to a moderate overloading of long chain esters on the investigated IL stationary phases, although their peak widths were in line with those of the other components.

*Watercol™ performance with a suspected allergen standard mixture* – A standard mixture of 29 compounds in the perfume field, 24 of them included in the list of EU suspected allergens was analyzed

with both columns, to evaluate 1460 and 1910 performances *versus* conventional and IL columns on analytes of routine interest in the field. Table 2 reports tailing factors and  $\sigma$  values, together with the area repeatability (n=3) of each allergen, on the two columns compared to SLB-IL60i, while Figure 2 shows the recovery of the analytes measured *versus* that of OV 1701, taken as reference, and compared to that of commercial SLB-IL 60 and SLB-IL 60i. Figure S1 reports the GC-FID profiles of the allergen standard mixture analyzed on IL60i, 1460, and 1910 columns. As a preliminary consideration, the results indicate that efficiency and inertness of both columns are good, while component recovery is comparable to that of SLB-IL 60, indicating the adsorption of some components.

*Watercol™ 1460* - With this column, three sample components (*p*-anisyl alcohol (**18**), cinnamyl alcohol (**20**), and vanillin (**24**)) were not detected, probably because of either irreversible adsorption and/or retention outside the time range of the analysis (120 min). Moderate peak distortion occurred for all substance with a free hydroxyl group in their structure (mainly alcohols and phenolic compounds) with tailing factors ranging between about 1.20 (eugenol (**15**)) and 1.34 (benzyl alcohol (**8**)). Only benzyl salicylate (**28**) showed a tailing factor of 1.6. Peak width was in general higher than that of the same components analyzed with IL60i, in particular for the early-eluting peaks, indicating the columns have lower efficiency. A further factor affecting efficiency is the minimum operative temperature of 1460, which is slightly higher than the initial temperature adopted for this analysis (40° vs. 60°C), as is clear from the  $\sigma$  value of limonene (**1**) (0.213). The same analysis carried out starting from 70°C resulted in a correct peak shape for limonene ( $\sigma$ : 0.041, tailing factor: 1.043). The initial temperature of 40°C was chosen so as to run all analyses under the same conditions, and to obtain comparable data. This consideration is also confirmed by the  $\sigma$  values of the late-eluting peaks, which are comparable and in some cases better than those of the corresponding peaks with IL60i. Area repeatability was very good, RSD% ranging from 0.78 for benzyl benzoate (**27**) to 5.38 for benzyl alcohol (**8**)

Lastly, the recovery percentage vs. OV-1701 was measured using methyl-eugenol (**5**) as internal reference for normalization, its peaks with both 1460 and 1910 being of comparable intensity, and of similar symmetry and width (Figure 2). Twelve components presented an adsorption above 35%; their normalized area was reduced to below 65% compared to their normalized area when analyzed with OV-1701, the most strongly adsorbed were benzyl alcohol (**8**), whose recovery was 22.1%, and the farnesols (**19a** and **19b**) at 8.7% and 17.6%, respectively. The 1460 column had inertness similar to that of IL 60, where several compounds were significantly adsorbed.

*Watercol™ 1910* – 1910 showed different selectivity from 1460 and had operative temperatures in the range 40° to 180°C. With this column, only one compound was not detected (vanillin (**24**)) and six coeluted (see table 2). Most peaks have satisfactory symmetry, falling in the range 0.8-1.2, without any apparent relationship to their specific structural characteristic(s) or function(s). Only eugenol (**15**) and benzyl cinnamate (**29**) presented highly asymmetrical peaks, with tailing factors of 2.46 and 2.64 respectively. Peak widths ( $\sigma$  values) were in many cases comparable or only slightly higher than those obtained with IL60i. Eugenol (**15**), benzyl salicylate (**28**), and benzyl cinnamate (**29**) showed very broad peaks (see above) and had  $\sigma$  values of 0.184, 0.153, and 0.289, respectively, probably because the operative temperature was too low, due to the thermal limits of the stationary phase. Area repeatability was very good: RSD% ranged from 0.34 for farnesol (**19b**) to 3.27 for benzyl cinnamate (**29**).

As for 1460, the percent recovery vs. OV 1701 was determined using methyl-eugenol (**5**) as internal reference for normalization (Figure 2). The 1910 column inertness with the allergen standard mixture was slightly better than that of IL60. Fourteen components presented relative adsorption above 35%, meaning that their recovery vs. OV 1701 is less than 65%. The lowest value was that of eugenol (**15**), whose recovery was 30.2%, in spite of its good shape and peak width.

### **Quali-quantitative analysis of real-world aqueous samples**

#### *Direct analysis of essential oil aqueous solutions with Watercol™ columns*

Many cosmetic preparations (lotions, tonics, perfumes, etc.) require essential oils to be solubilized in aqueous media. Essential oils are in general lipophilic, meaning that solubilization discriminates their components by polarity, which alters, among others, the relative ratios between hydrocarbons and oxygenated compounds in the final product, since the former are poorly soluble in water or ethanol/water solvents. In some cases, quality control of specific markers or biologically-active components is thus mandatory. Water-compatible IL stationary phases emphasize the general behavior of all IL in GC, i.e. they have a peculiar selectivity that affords very good discrimination between light hydrocarbons and oxygenated compounds; with the columns investigated here, hydrocarbons are poorly retained, mainly eluting in the region of the solvent, and not being well separated from one another, unlike what occurs with oxygenated compounds. Watercol™ columns can therefore be very useful for the direct analysis of essential oil aqueous solutions. Figure 3 reports the GC patterns obtained with 1460 (3a) and 1910 (3b) of the oxygenated fractions of lavender and peppermint essential oils, and of total tea-tree essential oil, obtained after direct injection of their 1:1 ethanol/water solutions.

*Watercol™ 1460 (Figure 3a)* – All oxygenated monoterpenoids of the three essential oils were very well separated. Sesquiterpene hydrocarbons also eluted in the same region of the chromatogram as the oxygenated compounds, as shown in the lavender (caryophyllene) and peppermint (caryophyllene, germacrene D) essential oil patterns. However, all oxygenated markers of both essential oils were base-line separated, affording correct characterization of the essential oil aqueous solutions; specifically, linalyl and lavandulyl acetates and linalool and lavandulol for lavender essential oil, and the four menthol isomers, the menthone isomers, menthyl acetate, pulegone, and menthofurane for peppermint essential oil. Tea-tree essential oil is characterized by 1,8-cineole, terpinen-4-ol and  $\alpha$ -terpineol. The latter two markers were very well separated, while 1,8-cineole could only partially be separated when applying an initial temperature of 40°C, since it elutes in the cluster of peaks of monoterpene hydrocarbons and solvent(s). However, the quantitation of terpinen-4-ol and  $\alpha$ -terpineol is of particular interest, since these compounds contribute to defining the origin of this essential oil, which is often the object of frauds, because of the higher quality and consequent higher cost of essential oils originating from Australia.

*Watercol™ 1910 (Figure 3b)* – The 1910 column was slightly less effective, although all oxygenated monoterpenoids of lavender and peppermint essential oils were base-line separated. In the tea-tree essential oil, 1,8-cineol coelutes with the solvent(s), but terpinen-4-ol and  $\alpha$ -terpineol were very well separated, making correct quality and origin control possible.

*Direct identification of suspected allergens in commercial perfumes* – Five commercial perfumes of different brands were purchased in a supermarket and analyzed directly as such, to detect and identify characterizing components and suspected allergens (see previous paragraph). Analyte identification was achieved by crossed comparison of the results obtained with the two Watercol™ columns investigated i) through their linear retention indices ( $I^T_s$ ) calculated vs. a standard mixture of C9-C30 hydrocarbons by GC-FID analysis, in combination with ii) their mass spectra obtained by GC-MS after HS-SPME sampling. Figure 4 and 5 report the GC-FID patterns of the five commercial perfumes and of the reference allergen standard mixture, together with the components–identified in them, with 1460 and 1910 columns, respectively.

*Direct quantitation of suspected allergens and markers in commercial perfumes* – The same perfumes were also submitted to true quantitation by external calibration with the pure standards, to quantify one characteristic component (linalyl acetate (**30**)) and two suspected allergens (linalool (**2**) and  $\alpha$ -ionone (**14**)) taken as markers. These compounds were chosen because they were present in most of the perfumes

investigated in different amounts. These analyses were also used to evaluate analytical performance of Watercols™ in terms of analyte linearity ( $R^2$ ), area repeatability, and intermediate precision, again to assess consistency of column behavior over time. The  $R^2$  values show that the linearity of both columns in the concentration range investigated was very good (i.e. always above 0.997 for 1460 and above 0.991 for 1910). Area repeatability was also very good with both IL columns, the maximum RSD% being 5.6% for linalyl acetate (**30**) with 1460 and 5.3% for linalool (**2**) for 1910. Intermediate precision for both columns was slightly lower (maximum RSD%: 8.4% for linalyl acetate (**30**) for 1460 and 9.3% for linalool on 1910). LOD and LOQ with both columns were in agreement with the leave-on suspected allergens limits, the highest LOD being 3 ppm (linalool (**2**) and  $\alpha$ -ionone (**14**)) for 1460 and 5 ppm (linalyl acetate (**30**)) for 1910, while LOQ was 7 ppm (linalool(**2**)) for 1460 and 9 ppm (linalyl acetate (**30**)) for 1910. Table 3 reports the figures of merit of the method applied. With 1910 in the analysis of perfume 4, linalyl acetate (**30**) coeluted with another component, thus altering the quantitative results (Figure 5 and Table 4).

The results of these analyses were compared and confirmed by quantifying the same analytes in the same perfumes with the official EU method [12, 13]. The results with the two methods were fully comparable, showing that both 1460 and 1910 can successfully be used to quantify target analytes with Watercol™ columns directly, without dilution (Table 4).

## Conclusions

The results show that water-compatible gas chromatographic stationary phases based on ILs open new perspectives in the analytical and bioanalytical field, thanks to the fact that they can be applied routinely to the direct analysis of samples with water as main solvent. The peculiar selectivity of water-compatible IL columns can be applied to aqueous or hydroalcoholic samples, making them very promising, in particular for the fragrance and essential oil fields. The use of water-compatible IL columns in routine analysis in quality control laboratories means that time-consuming sampling procedures to transfer fractions or analytes of interest to solvents compatible with conventional columns can be avoided or simplified.

However, the results with samples where water is the main solvents show that these IL columns still present a slightly lower efficiency and higher activity than inert IL columns meaning that significant efforts must still be made to achieve their full application in quality control. Further progresses are expected in column manufacturing, to improve their range of operative temperatures (where possible), their inertness and reduce their activity, exactly as it has occurred for the first generation of conventional IL columns, which recently resulted in the introduction of the inert series [11]. Moreover, in routine analysis it is very

often necessary that gas chromatography adopts mass spectrometry as detector: further experiments are under way to evaluate the effects of the introduction of aqueous samples on MS performance.

### **Acknowledgements**

The authors are indebted to Supelco (Bellefonte, PA, USA) for providing the ionic liquid columns, to Dr. Len Sidisky (Supelco, Bellefonte, PA, USA) for helpful discussion and advice, and to Robertet SA (Grasse, France) for financial support to the laboratory.

*The authors have declared no conflicts of interest*

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**Captions to figures:**

**Figure 1** – GC-TCD profiles of a 1:1 ethanol:water standard mixture analyzed with the two Watercol™ columns.

**Figure 2.** Recovery of suspected allergens, calculated from the normalized absolute area of each compound with each investigated IL *versus* OV-1701 columns, taken as reference.

**Figure 3:** 1460 (a) and 1910 (b) GC patterns of the oxygenated fractions of lavender and peppermint essential oils, and that of total tea-tree essential oil

**Figure 4** – GC-FID profiles of commercially-available perfumes and allergen standard mixture with Watercol 1460 column. Peak identification: 1: limonene, 2: linalool, 3: estragole, 4: phenylacetaldehyde, 5: methyl 2-octynoate, 6: citronellol, 7: geraniol, 8: benzyl alcohol, 9: neral, 10: geranial, 11:  $\alpha$ -isomethyl ionone, 12: methyl eugenol, 13: hydroxycitronellal, 14:  $\alpha$ -ionone, 15: eugenol, 16: linal, 17: cinnamaldehyde, 18: anisyl alcohol, 19: farnesol isomers, 20: cinnamyl alcohol, 21: amyl cinnamaldehyde, 22: hexyl cinnamaldehyde, 23:  $\alpha$ -pentylcinnamyl alcohol, 24: vanillin, 25: lylal isomers, 26: coumarin, 27: benzyl benzoate, 28: benzyl salicylate, 29: benzyl cinnamate, 30: linalyl acetate.

**Figure 5** – GC-FID profiles of commercially-available perfumes and allergen standard mixture with Watercol 1910 column. Peak identification: see figure 4.



## Captions to tables

**Table 1** - Retention times, tailing factors peak widths ( $\sigma$ ) and recovery vs. IL60i of the Grob test components when analyzed on Watercol 1460, Watercol 1910 and SLB-IL60i. *N.D.*: Not Detected; *N.M.*: Not Measured

**Table 2** Tailing factors and  $\sigma$  values together with repeatability of each component of the allergen standard mixture investigated on 1460 and 1910 columns compared to SLB-IL60i. *N.D.*: Not Detected.

**Table 3** – Figures of merit of the quantitative method applied to Linalool (**2**), Linalyl acetate (**30**) and  $\alpha$ -ionone (**11**).

**Table 4** – Concentration of Linalool (**2**), Linalyl acetate (**30**) and  $\alpha$ -ionone (**14**) in five commercial perfumes after analysis on Watercol 1460, Watercol 1910 and OV 1701 columns. *N.D.*: Not Detected

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	Ret. Time			Tailing factor			$\sigma$ (min)			Recovery (%)	
	IL60i	Watercol 1460	Watercol 1910	IL60i	Watercol 1460	Watercol 1910	IL60i	Watercol 1460	Watercol 1910	Watercol 1460	Watercol 1910
1-Octanol	21.97	23.85	16.87	1.46	1.136	1.227	0.042	0.047	0.049	100.0	100.0
2,3-Butanediol	<i>N.D.</i>	28.22	32.72	<i>N.D.</i>	1.847	1.541	<i>N.D.</i>	0.060	0.134	<i>N.M.</i>	<i>N.M.</i>
2,3-Dimethylphenol	38.01	39.46	32.27	0.962	1.108	0.967	0.040	0.035	0.037	80.2	96.1
3,5-Dimethylaniline	34.85	34.58	31.25	0.986	1.034	0.995	0.038	0.035	0.039	123.3	115.8
Decane	3.80	3.01	2.15	1.043	<i>N.M.</i>	1.134	0.016	<i>N.M.</i>	0.015	<i>N.M.</i>	93.2
Dicyclohexylamine	59.42	21.48	<i>N.D.</i>	5.685	2.00	<i>N.D.</i>	0.818	0.151	<i>N.D.</i>	85.0	0.0
Dodecane	8.64	5.58	2.97	0.942	1.049	0.931	0.025	0.174	0.021	88.6	79.8
Hexanoic acid, 2-ethyl-	36.89	43.95	<i>N.D.</i>	1.305	2.61	<i>N.D.</i>	0.239	0.098	<i>N.D.</i>	564.5	<i>N.M.</i>
Methyl decanoate	29.27	20.03	12.75	0.937	1.07	0.937	0.033	0.056	0.058	101.2	64.7
Methyl undecanoate	34.33	23.09	15.08	0.908	1.032	0.839	0.034	0.045	0.060	97.1	66.3
Methyldodecanoate	39.174	26.00	17.45	0.896	1.018	0.686	0.034	0.037	0.073	93.0	75.1

		Ret. Time			Tailing factor			$\sigma$ (min)			Repeatability (n=3)	
		IL60i	W.C. 1460	W.C. 1910	IL60i	W.C. 1460	W.C. 1910	IL60i	W.C. 1460	W.C. 1910	W.C. 1460	W.C. 1910
1	Limonene	5.007	5.504	4.542 - <i>Coelution</i>	1.071	1.06	<i>Coelution</i>	0.017	0.213	<i>Coelution</i>	1.25	0.89
2	Linalool	12.444	25.305	22.599	1.008	1.057	0.995	0.025	0.078	0.041	2.31	0.51
3	Estragole	18.473	27.839	25.68	0.999	1.03	0.926	0.028	0.056	0.040	4.59	1.45
4	Phenylacetaldehyde	19.64	31.764	<i>Coelution 1</i>	1.23	1.293	<i>Coelution 1</i>	0.040	0.060	<i>Coelution 1</i>	4.58	1.14
5	2-Octynoic acid, methyl ester	20.221	27.304	24.778	0.965	1.006	0.885	0.029	0.064	0.042	1.94	1.27
6	Citronellol	20.447	37.195	<i>Coelution 2</i>	1.071	0.997	<i>Coelution 2</i>	0.031	0.049	<i>Coelution 2</i>	4.36	1.46
7	Geraniol	22.31	40.303	<i>Coelution 1</i>	1.055	1.199	<i>Coelution 1</i>	0.032	0.046	<i>Coelution 1</i>	3.10	1.14
8	Benzyl alcohol	23.01	48.303	51.087	1.078	1.342	0.979	0.035	0.042	0.045	5.38	1.10
9	Neral	23.617	31.602	31.869	0.989	0.983	1.049	0.031	0.064	0.043	0.79	3.24
10	Geranial	25.053	33.689	34.005	0.997	1.047	1.003	0.031	0.057	0.041	0.89	1.66
11	$\alpha$ Iso-Methyl-ionone	29.857	37.466	<i>Coelution 2</i>	0.97	1.018	<i>Coelution 2</i>	0.032	0.077	<i>Coelution 2</i>	2.19	1.46
12	Methyl eugenol	32.403	46.408	47.651	0.991	0.984	0.909	0.032	0.038	0.037	1.86	0.78
13	Hydroxy citronellal	32.55	51.205	51.953	0.995	1.033	0.85	0.033	0.040	0.036	1.96	1.58
14	$\alpha$ -Ionone	32.866	41.333	35.84	1.066	1.039	0.931	0.031	0.055	0.041	4.25	2.11
15	Eugenol	33.213	58.056	70.32	1.02	1.316	2.459	0.034	0.042	0.184	1.65	1.94
16	Lilial	33.972	45.967	43.398	1.004	0.973	1.132	0.032	0.048	0.098	4.92	1.49
17	Cinnamaldehyde	34.606	52.437	55.646	0.997	1.048	0.951	0.038	0.043	0.045	4.45	0.86
18	p-Anisyl alcohol	37.148	<i>N.D.</i>	74.003	1.013	<i>N.D.</i>	0.997	0.041	<i>N.D.</i>	0.064	<i>N.D.</i>	1.15
19a	Farnesol isomer a	36.814	56.923	50.506	0.999	1.31	0.955	0.031	0.043	0.035	5.31	0.69
19b	Farnesol isomer b	36.981	57.765	51.322	0.989	1.27	0.934	0.032	0.045	0.034	4.89	0.34
20	Cinnamyl alcohol	37.759	<i>N.D.</i>	71.474	1.021	<i>N.D.</i>	0.96	0.048	<i>N.D.</i>	0.062	<i>N.D.</i>	0.60
21	Amyl-cinnamaldehyde	40.922	56.463	50.838	1.282	0.894	0.797	0.051	0.040	0.041	1.89	0.97
22	$\alpha$ -Hexyl-cinnamaldehyde	43.728	59.533	52.741	0.973	0.961	0.81	0.033	0.038	0.041	2.34	1.45
23	$\alpha$ -Pentyl-cinnamyl alcohol	44.049	64.191	66.651	1.228	1.322	0.873	0.038	0.039	0.050	1.49	0.87
24	Vanillin	45.853	<i>N.D.</i>	<i>N.D.</i>	1.395	<i>N.D.</i>	<i>N.D.</i>	0.040	<i>N.D.</i>	<i>N.D.</i>	<i>N.D.</i>	<i>N.D.</i>
25a	Lyril isomer a	46.424	68.792	<i>Coelution 3</i>	0.879	1.148	<i>Coelution 3</i>	0.038	0.040	<i>Coelution 3</i>	4.37	1.13
25b	Lyril isomer b	46.817	69.2	<i>Coelution 3</i>	0.979	1.241	<i>Coelution 3</i>	0.036	0.041	<i>Coelution 3</i>	3.25	1.13

26	Coumarin	49.251	71.153	82.705	0.984	0.932	0.90	0.047	0.043	0.078	5.12	1.05	<b>Table 2 -</b>
27	Benzyl benzoate	49.639	70.157	71.474	0.982	0.956	0.96	0.037	0.037	0.062	0.78	1.59	
28	Benzyl salicylate	51.894	74.287	80.034	1.017	1.602	1.033	0.041	0.051	0.153	1.02	0.67	
29	Benzyl cinnamate	61.776	86.691	85.85	0.998	1.041	2.637	0.044	0.038	0.289	1.13	3.27	

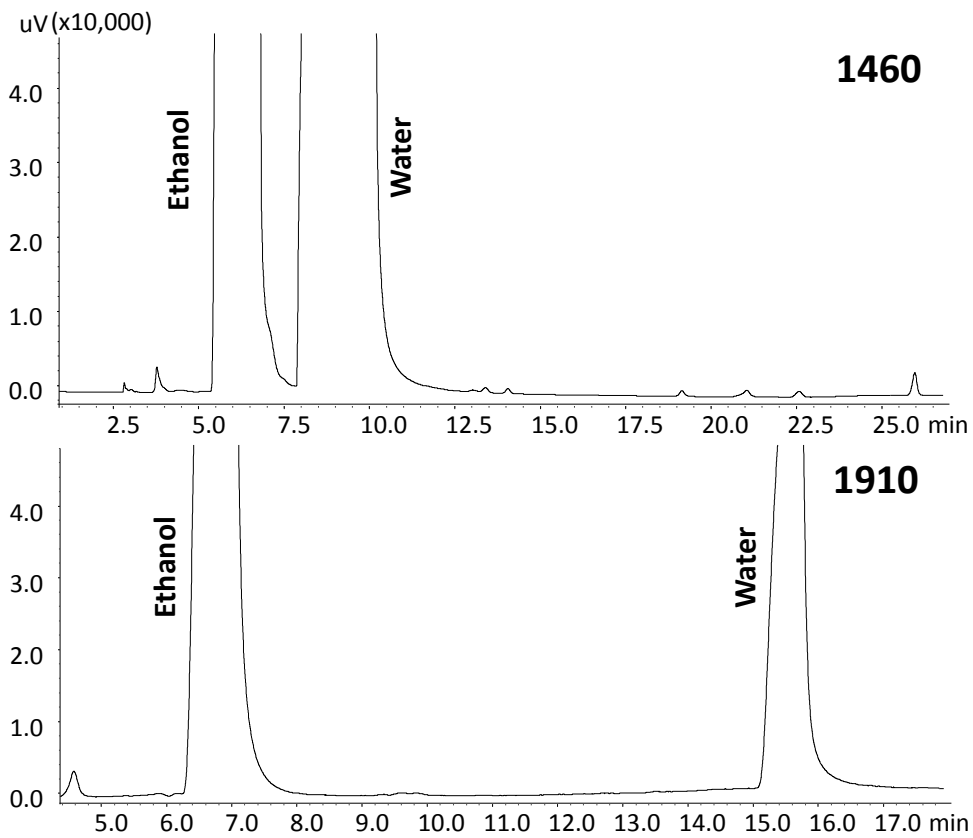
Tailing factors and  $\sigma$  values together with area repeatability of each component of the investigated allergen standard mixture on 1460 and 1910 columns compared to SLB-IL60i. *N.D.*: Not Detected.

**Table 3** – Figures of merits of the quantitative method applied to Linalool (**2**), Linalyl acetate (**30**) and  $\alpha$ -Ionone (**14**).

Col.	Analyte	Linear range investigated (mg L <sup>-1</sup> )	Slope $\pm$ error	Linearity (R <sup>2</sup> )	LOD (mg L <sup>-1</sup> )	LOQ (mg L <sup>-1</sup> )	Repeatability % RSD 50 mg L <sup>-1</sup> (n=5)	Intermediate precision % RSD 50 mg L <sup>-1</sup> (n=4)
1460	Linalool ( <b>2</b> )	50-5000	172.73 $\pm$ 6.93	0.997	3	7	5.2	7.9
	Linalyl acetate ( <b>30</b> )	50-5000	146.29 $\pm$ 5.54	0.997	2	5	5.6	8.4
	$\alpha$ -Ionone ( <b>14</b> )	50-2000	146.98 $\pm$ 4.30	0.999	3	6	3.4	6.1
1910	Linalool ( <b>2</b> )	50-5000	183.41 $\pm$ 7.01	0.994	4	7	5.3	9.3
	Linalyl acetate ( <b>30</b> )	50-5000	161.15 $\pm$ 4.87	0.991	3	9	4.9	8.7
	$\alpha$ -Ionone ( <b>14</b> )	50-2000	137.58 $\pm$ 5.26	0.998	5	8	4.3	7.6

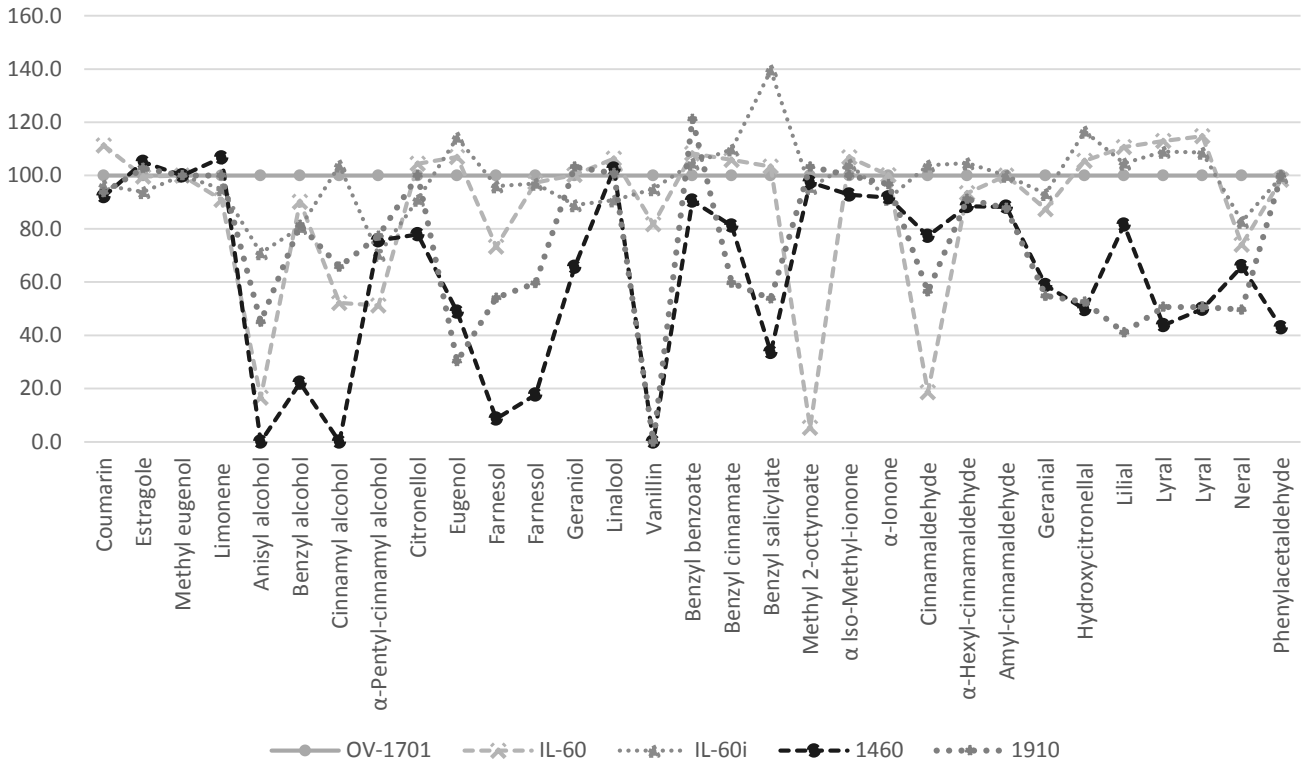
**Table 4** – Concentration of Linalool (**2**), Linalyl acetate (**30**) and  $\alpha$ -Ionone (**14**) in five commercial perfumes after analysis on Watercol 1460, Watercol 1910 and OV 1701 columns. *N.D.*: Not Detected

Analyte		Absolute amount (mg L <sup>-1</sup> )		
		Column 1460	Column 1910	Official method [12, 13]
Perf. 1	Linalool ( <b>2</b> )	4314 (40)	4745 (15)	4413 (22)
	Linalyl acetate ( <b>30</b> )	3772 (28)	3971 (32)	3312 (35)
	$\alpha$ -Ionone ( <b>14</b> )	912(6)	869 (5)	841 (8)
Perf. 2	Linalool ( <b>2</b> )	419 (3)	450 (0.04)	439 (3)
	Linalyl acetate ( <b>30</b> )	751 (7)	767 (0.09)	738 (6)
	$\alpha$ -Ionone ( <b>14</b> )	125 (1)	94 (0.01)	103 (2)
Perf. 3	Linalool ( <b>2</b> )	1947 (12)	1989 (23)	2012 (19)
	Linalyl acetate ( <b>30</b> )	<i>N.D.</i>	<i>N.D.</i>	<i>N.D.</i>
	$\alpha$ -Ionone ( <b>14</b> )	404 (2)	Coelution	380 (4)
Perf. 4	Linalool ( <b>2</b> )	2798 (16)	Coelution	2846 (13)
	Linalyl acetate ( <b>30</b> )	4653 (39)	4782 (41)	4568 (38)
	$\alpha$ -Ionone ( <b>14</b> )	<i>N.D.</i>	<i>N.D.</i>	<i>N.D.</i>
Perf. 5	Linalool ( <b>2</b> )	3893 (37)	4124 (41)	4073 (36)
	Linalyl acetate ( <b>30</b> )	4667 (51)	4918 (49)	4505 (52)
	$\alpha$ -Ionone ( <b>14</b> )	<i>N.D.</i>	<i>N.D.</i>	<i>N.D.</i>



**Figure 1** – GC-TCD profiles of a 1:1 ethanol:water standard mixture analyzed with the two Watercol™ columns.

## Recovery (%)



**Figure 2.** Recovery of suspected allergens, calculated from the normalized absolute area of each compound with each investigated IL *versus* OV-1701 columns, taken as reference.



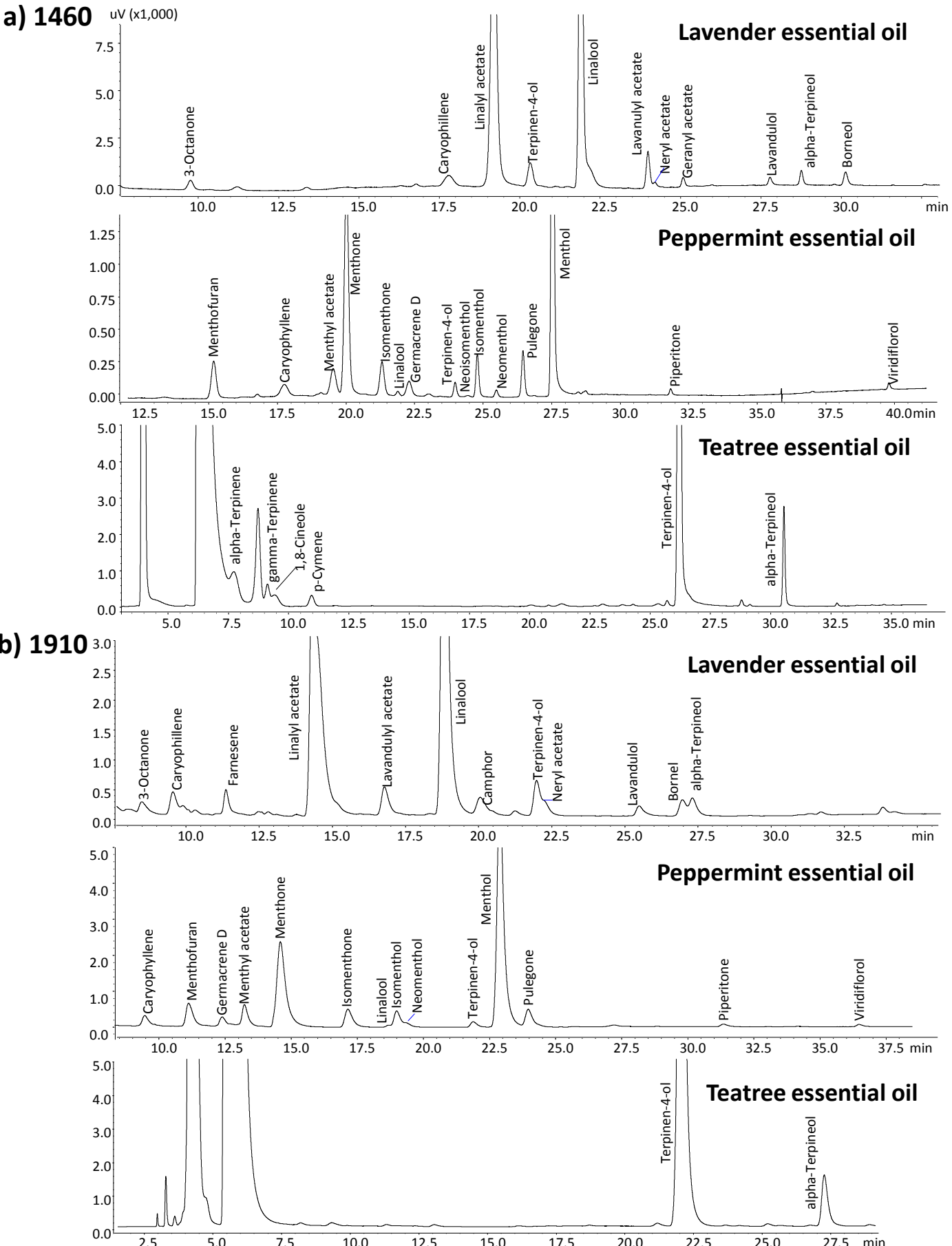
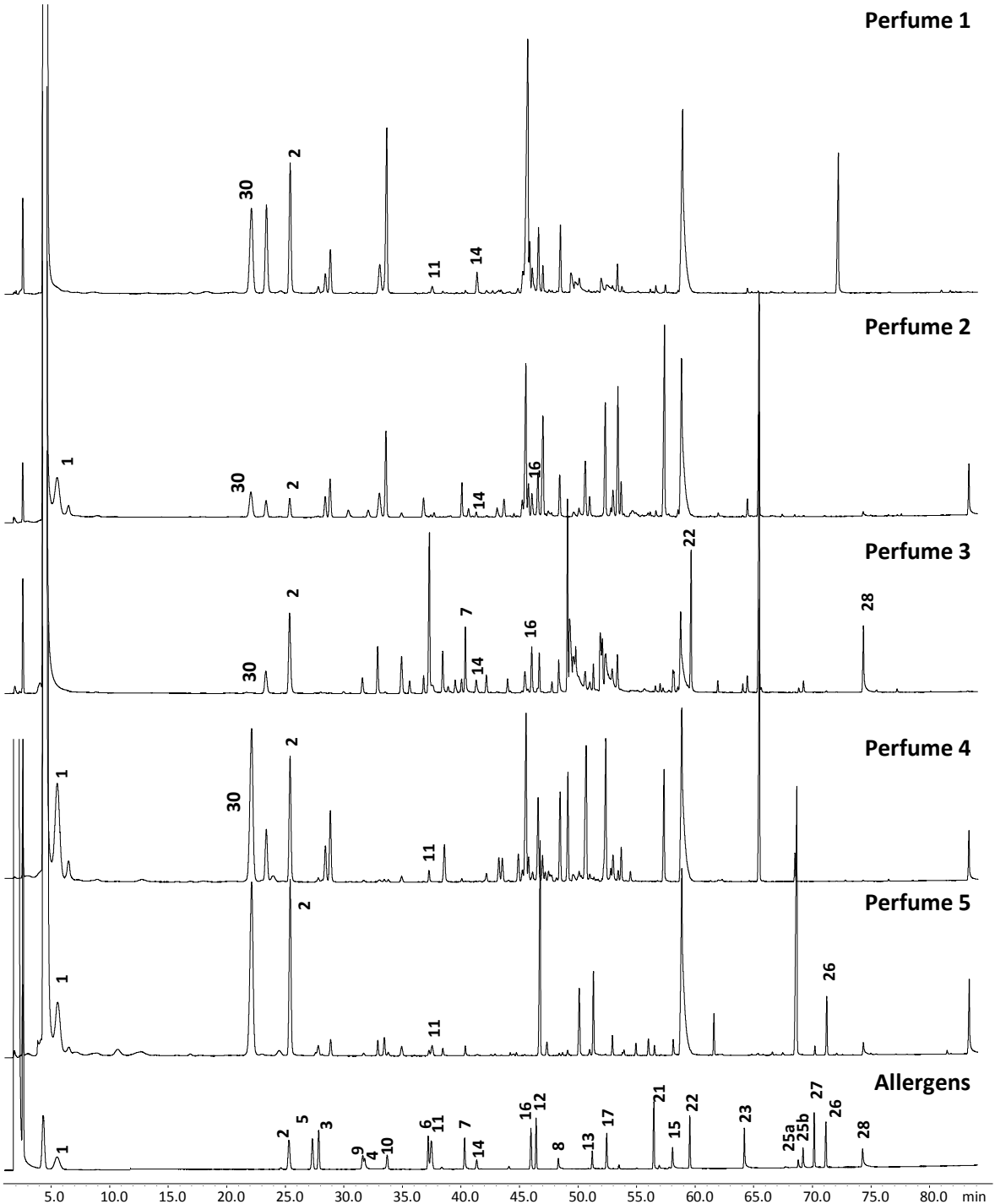
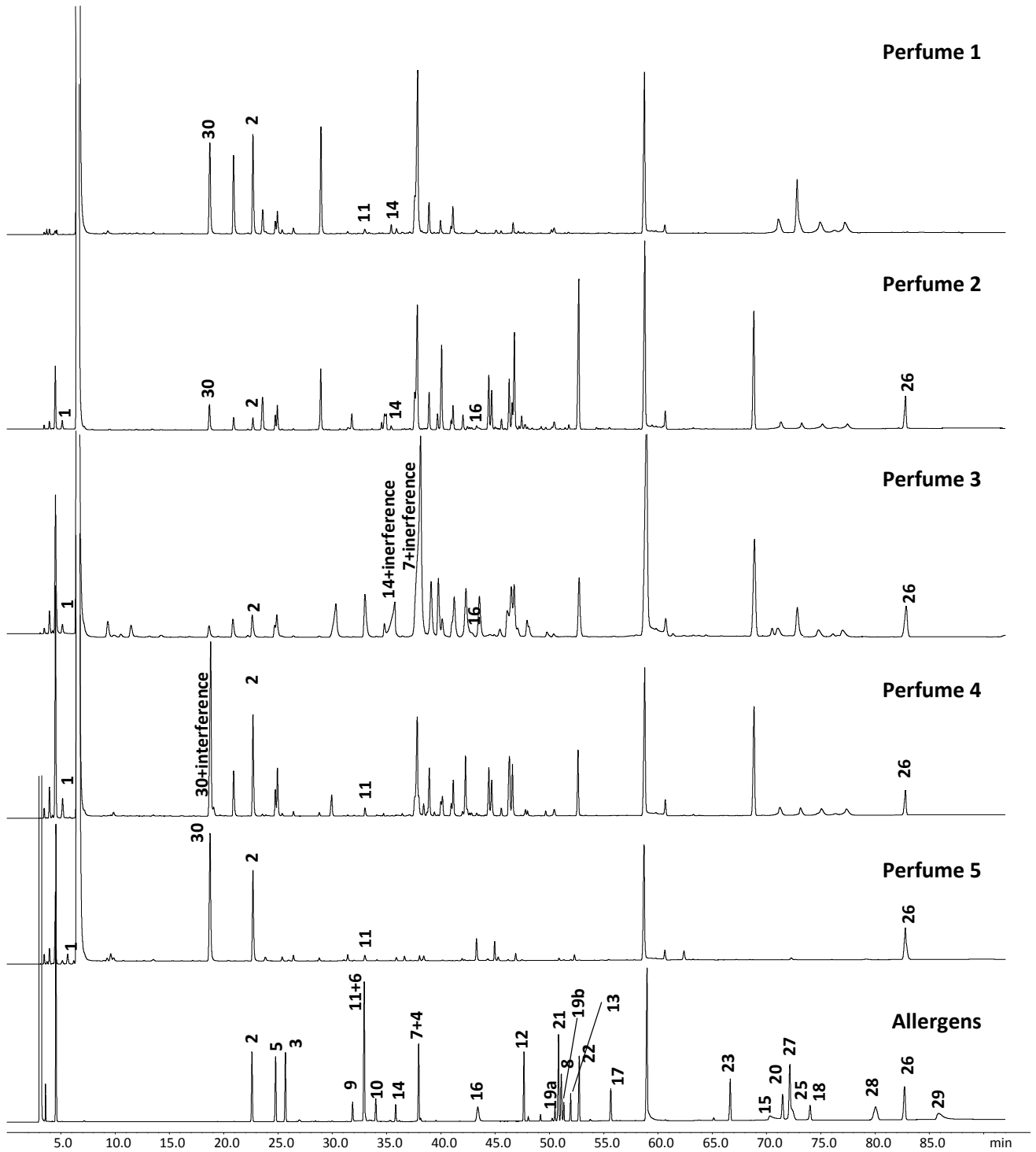


Figure 3: 1460 (a) and 1910 (b) GC patterns of the oxygenated fractions of lavender and peppermint essential oils, and that of the total tea tree essential oil.



**Figure 4** – GC-FID profiles of commercially-available perfumes and allergen standard mixture with Watercol 1460 column.. Peak identification: 1: limonene, 2: linalool , 3: estragole, 4: phenylacetaldehyde, 5: methyl 2-octynoate, 6: citronellol, 7: geraniol, 8: benzyl alcohol, 9: neral, 10: geranial, 11:  $\alpha$ -isomethyl ionone, 12: methyl eugenol, 13: hydroxycitronellal, 14:  $\alpha$ -ionone, 15: eugenol, 16: lilial, 17: cinnamaldehyde, 18: anisyl alcohol, 19: farnesol isomers, 20: cinnamyl alcohol, 21: amyl cinnamaldehyde, 22: hexyl cinnamaldehyde, 23:  $\alpha$ -pentylcinnamyl alcohol, 24: vanillin, 25: lylal isomers, 26: coumarin, 27: benzyl benzoate, 28: benzyl salicylate, 29: benzyl cinnamate, 30: linalyl acetate.

1910



**Figure 5** – GC-FID profiles of commercially-available perfumes and allergen standard mixture with Watercol 1910 column. Peak identification: see figure 4.

# Analytical and Bioanalytical Chemistry

## Supplementary material

### Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils.

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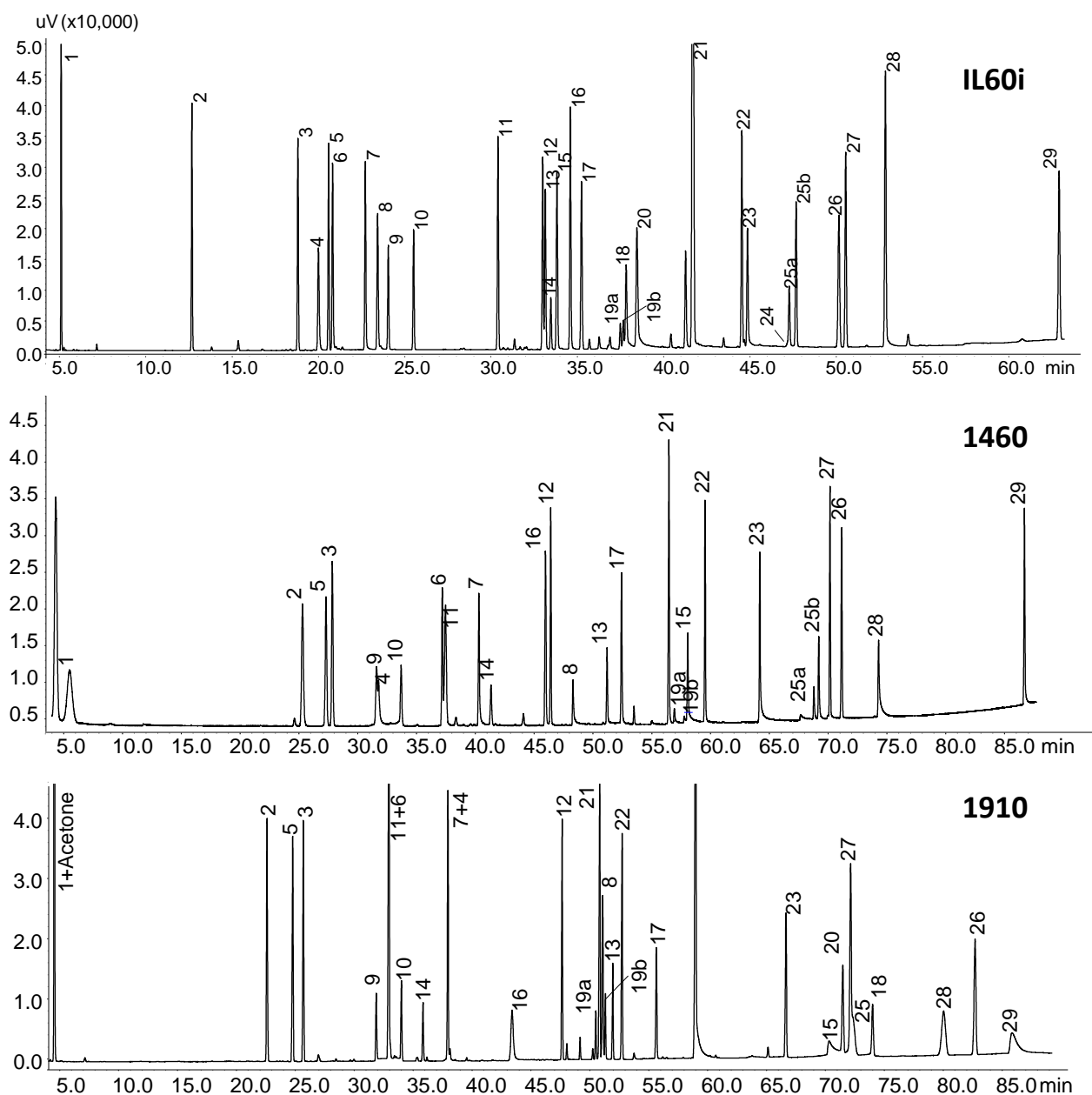
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**Figure S1** – Comparison of the GC-FID profiles of the allergen standard mixture obtained on IL60i and the 1460 and 1910 columns. Peak identification: 1: limonene, 2: linalool, 3: estragole, 4: phenylacetaldehyde, 5: methyl 2-octynoate, 6: citronellol, 7: geraniol, 8: benzyl alcohol, 9: neral, 10: geranial, 11:  $\alpha$ -isomethyl ionone, 12: methyl eugenol, 13: hydroxycitronellal, 14:  $\alpha$ -ionone, 15: eugenol, 16: linal, 17: cinnamaldehyde, 18: anisyl alcohol, 19: farnesol isomers, 20: cinnamyl alcohol, 21: amyl cinnamaldehyde, 22: hexyl cinnamaldehyde, 23:  $\alpha$ -pentylcinnamyl alcohol, 24: vanillin, 25: lylal isomers, 26: coumarin, 27: benzyl benzoate, 28: benzyl salicylate, 29: benzyl cinnamate.