Room temperature ionic liquids: new GC stationary phases with a novel selectivity for flavour and fragrance analyses

Room temperature ionic liquids: new GC stationary phases with a novel selectivity for flavour and fragrance analyses

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Abstract

Ionic liquids (ILs) are of great interest as moderately-polar to polar stationary phases for GC, because their selectivity differs markedly from that of conventionally-used phases. In the flavour, fragrance and essential oil fields, analysts often deal with complex mixtures of compounds having similar structural and physical characteristics (e.g. mono- and sesquiterpenoids), therefore requiring an interactive combination between chromatographic and mass spectral data for correct identification. New GC stationary phases with different selectivity must therefore be continually tested.

Performance and evolution over time of commercially-available IL columns *versus* those commonly used in these fields are here evaluated, mainly in view of their routine use. Chromatographic and separative properties (efficiency, separation capability, inertness and/or activity) of commercially-available IL columns were compared to those of columns coated with 5%phenyl-95%methylpolysiloxane, 14%cyanopropyl-86%polysiloxane, and polyethylene glycol, on different complexity samples, including standard mixtures of volatile suspected allergens and pesticides, and cornmint and vetiver essential oils.

The results show that IL columns can successfully be used for a wide range of applications characteristic of these fields, mainly because of their unusual selectivity, in particular when separations based on functional groups are required. Moreover, the latest generation of IL columns (IL61 and IL60) presents chromatographic performance comparable to or only slightly lower than that of the conventional columns routinely used in these fields.

Keywords: Ionic liquids, GC chromatographic properties, selectivity, allergen, pesticides, essential oils.

1. INTRODUCTION

Room-temperature ionic liquids (ILs) are a class of organic nonmolecular solvents that are liquid at 20°C; in general they consist of an organic cation containing nitrogen or phosphorus (e.g. alkyl imidazoniumphosphonium) and an organic or inorganic anion. Low volatility, an in general high thermal stability (over 300°C), negligible vapour pressure, high viscosity and good wetting abilities on the inner wall of fused silica capillaries make them suitable as GC stationary phases.

Several studies have been carried out on new IL derivatives and their retention mechanism [1-3], since their introduction as stationary phases for capillary GC [4-6], due to their ability to be tuned in terms of polarity and selectivity. Selectivity is the main object of interest, since it makes their separation capability different from that of conventional and popular stationary phases, for instance, those based on polysiloxanes and polyethylene glycol. Separative differences are mainly due to their dual-nature retention mechanism (i.e. partition and interfacial adsorption or their combination) [7-9], which enables them to separate both polar and non-polar compounds, thus extending their use to analytes within a wide range of polarity. Available IL applications have recently been reviewed [1-3], and cover several fields, including fatty acids and related esters, petrochemicals, essential oils and flavours and fragrances, environmental samples and pesticides, and polycyclic aromatic hydrocarbons. In the flavour and fragrance field, IL columns have already been used for the analysis of flavour and fragrance mixtures [10-11] , allergens [12] and some essential oils (i.e. lemon essential oil [13] and fennel, cinnamon and nutmeg essential oils [14]). The first commercial IL column for gas chromatography was introduced in 2008 with the acronym SLB-IL100 [15,16]. It was a non-bonded column coated with 1,9-di(3-vinyl-imidazolium)-nonanebis (trifluoromethyl) sulfonylimidate with a maximum operative temperature of 230°C. Other columns with different polarity and characteristics were subsequently introduced; the chemical composition of the stationary phases of these columns is not described, but is a trademark of the manufacturer. They are distinguished on the basis of their increasing polarity numbers, calculated taking SLB-IL100 as reference [16] (SLB-IL59 [13], SLB-IL76, SLB-IL82 [17], SLB-IL111 [18]). More recently, new columns similar in polarity to SLB-IL59 but with an improved inertness have been introduced, in particular SLB-IL61 [19], and then SLB-IL60.

Analyses in the flavour, fragrance and essential oil fields must meet a very wide range of demands, from quality control to marker identification and quantitation, from detection of potentially toxic compounds (e.g. suspected allergens regulated by the European Commission) [20], to pesticide residues; thus such analyses involve analytes with highly different chromatographic behaviours. Samples in these fields are, in general, complex mixtures often consisting of isomeric components with similar structural and physical characteristics (e.g. mono- and sesquiterpenoids), thus being difficult to identify because their mass spectra are very similar and sometimes indistinguishable. As a consequence, diagnostic chromatographic data (e.g. retention indices [21]) become indispensable for correct analyte identification, which, to be reliable, in general requires an

interactive combination between the analyte retention indices on two different-polarity or -selectivity GC stationary phases and the mass spectrum. A continual search for new stationary phases with different selectivity and, at the same time, with good chromatographic properties in terms of efficiency and inertness, is therefore necessary to obtain separation patterns different from those of the currently-used polysiloxane and polyethylene glycol derivatives (for brevity's sake, from here on called "conventional" derivatives).

This study reports an evaluation of performance and evolution over time of commercially-available IL columns, mainly in view of their routine use in the flavour, fragrance and essential oil fields. In particular, it compares the chromatographic and separative effectiveness of the IL columns now available with those of conventional columns coated with 5%phenyl-95%methylpolysiloxane (SE-52); 14%cyanopropyl-86%polysiloxane (OV-1701); and polyethylene glycol (PEG) on two standard mixtures of suspected allergens and pesticides, and two essential oils of different complexity, cornmint and vetiver, selected as being representative of some routine applications in these fields.

2. EXPERIMENTAL

2.1 Samples and chemicals

The allergens standard mixture consisted of 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: α-isomethyl ionone (CAS: 15789-90-9), 12: methyl eugenol (CAS: 93-15-2), 13: hydroxycitronellal (CAS: 107-75-5), 14: α-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: α-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), 29: benzyl cinnamate (CAS: 103-41-3). They were solubilized in toluene at a concentration of 500µg/L. The pesticide standard mixture contained eight compounds: 1: chlordane cis (CAS:5103-71-9), 2: chlorpyriphos (CAS: 2921-88-2), 3: fonofos (CAS: 944-22-9), 4: heptachlor (CAS: 76-44-8), 5: hexaconazole (CAS: 79983-71-4), 6: metalaxyl (CAS: 57837-19-1), 7: propiconazole isomers (CAS: 60207-90-1), 8: vinclozolin (CAS: 50471-44-8). They were dissolved in toluene at a concentration of 100 µg/L. Commercial cornmint (*Mentha arvensis* L.) and vetiver (*Chysopogon zizanioides* (L.) Roberty ex *Vetiveria zizanioides* L.) essential oils were diluted 1:200 in cyclohexane before analysis. The hydrocarbon and oxygenated fractions of the above vetiver essential oil were obtained by fractionating 300 mg by column chromatography on silica gel; the hydrocarbon fraction was eluted with petroleum ether 100% and the oxygenated fraction with

petroleum ether/ethyl acetate 90/10 [22]. The fractions were then suitably diluted 1:200 in cyclohexane before analysis. Solvents were all HPLC grade from Riedel-de Haen (Seelze, Germany).

2.2 Analysis conditions

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

Columns: GC analyses were carried out with three 30 m × 0.25 mm d_c × 0.25 µm d_f conventional columns coated with SE-52, OV-1701, and PEG, and seven 30 m × 0.25 mm d_c× 0.20 µmd_f ionic liquid (IL) columns of different polarity and inertness (i.e. SLB-IL59 (IL59), SLB-IL60 (IL60), SLB-IL61 (IL61), SLB-IL76 (IL76), SLB-IL82 (IL82), SLB-IL100 (IL100) and SLB-IL111 (IL111)) All conventional and IL columns were provided by Supelco (Milan, Italy).

GC-MS conditions: Temperatures: injector: 270°C, transfer line: 290°C; ion source: 200°C; carrier gas: He, flow control mode: constant linear velocity: 36.1 cm/s. Temperature program: from 40°C (1min) to 270°C (2min) at 3°C/min for all columns with the exception of IL100, for which the final temperature was 230°C. 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.

GC-FID conditions: Temperatures: injector: 270°C, detector: 290°C; carrier gas: H_2, flow control mode: constant linear velocity: 35.3 cm/s. Temperature program and injection conditions: see GC-MS section. FID sampling rate: 40ms.

3. RESULTS AND DISCUSSION

In this section, the performance of the available IL columns is evaluated on the basis of their efficiency, separation capability, inertness and/or activity, by determining average peak width (σ) and asymmetry, adsorption and separation measure (Δs) [23], when applied to the analyses of standard mixtures of both suspected allergens and pesticides, and of cornmint and vetiver essential oils. The results are also compared to those of commonly-used columns, namely SE-52, OV-1701 and PEG.

3.1. Analysis of a suspected volatile allergen standard mixture

The standard mixture of 29 allergens (belonging to different chemical classes and covering a wide range of volatility and polarity) was first analyzed with IL and conventional columns. Different selectivity in allergen separation is fundamentally important in perfume analysis, where in general allergens are present together with dozens of other components.

The initial screening with the IL columns under investigation indicated IL59, IL82, IL61 and IL60 as the columns to be considered for this standard mixture, and OV-1701 as the conventional column to be taken as reference, because of its very high efficiency and inertness. Conversely, the chromatographic efficiency of IL76 was too low, the peak widths being at least double those of the above columns (data not shown); it was thus discarded for this analysis; IL100 and IL111 columns were not considered, because of their very high polarity, resulting in insufficient retention of volatile apolar components (e.g. monoterpene hydrocarbons).

Figure 1 reports the GC patterns of the allergen standard mixture with the columns investigated; peak width and tailing factors of the components of the allergen standard mixture were first determined for these columns. Table 1 reports average peak widths, tailing factors, and number of asymmetric peaks (i.e. peaks with tailing factors above 1.1 or below 0.9), and number of adsorbed peaks (see below). From these results, the following considerations can be made: a) the average peak width of IL59 was much higher than all other columns (σ: 3.72 s) and in particular than OV-1701 (σ: 1.86 s) and all peaks were asymmetrical; b) average peak width and number of asymmetric peaks of IL82 were lower than those of IL59; however, its average tailing factor was decidedly higher than that of either IL59 or OV-1701 (1.537 *vs*. 1.214 and 0.931 respectively), in particular for alcohols, e.g. 2.2 for benzyl alcohol (8) and 3.0 for α-pentyl-cinnamyl alcohol (23); c) the performance of IL61 was decidedly better: peak width was quite close to that of OV-1701 (σ: 2.28 *vs*. 1.86 s) while the number of asymmetric peaks dropped to 17 (instead of 25 for IL82 and 32 for IL59); d) IL 60 performed similarly to OV-1701, with average peak width equal to that of IL61 but a better average tailing factor, at 0.993, and only 10 asymmetric peaks (only some alcohols and phenyl acetaldehyde were slightly asymmetric).

Finally, adsorption was evaluated for all IL columns. This parameter was calculated by dividing the absolute area of each compound, determined with the investigated IL column, by that with OV-1701, taken as reference because of its high inertness. As is clear from Table 1 and Figure 2, which respectively report the number of compounds with adsorption exceeding 10% *vs* OV-1701, and the adsorption of each component, the inertness of the previous generation of IL columns is critical, in particular with alcohols and polar compounds, in some cases giving rise to humps rather than peaks (e.g. Figure 1, IL59 GC pattern). For IL61 and IL60, the number of adsorbed components and the relative adsorption values decreased very markedly.

The separation capability of IL columns was also determined, through the separation measure. *Δs*, defined as the number of consecutive non-overlapping σ-intervals within an arbitrary time interval ($t_b - t_a$) [23] was calculated for the 29 suspected allergens with the investigated IL columns. It was found to be higher than that of conventional columns (OV-1701: 1582), and increased from IL59 (1516) to IL82 (1787), indicating the ability of these columns to separate a greater number of compounds.

Although having good selectivity and separation power, IL82 does not present high chromatographic efficiency and inertness. IL60 should be preferred over IL61 for suspected allergen analysis, because of its better selectivity and chromatographic properties.

3.2. Analysis of a pesticide standard mixture

A standard mixture of eight pesticides belonging to different chemical classes (organochlorine, organophosphorous, triazole, phenylamides, dicarboxyimides) was analyzed to evaluate the IL columns performance in separating relatively low volatility and polar model molecules, containing groups or organic functions potentially interacting with IL stationary phases, and requiring higher temperatures for their GC analysis.

Figure 3 reports the GC patterns of the pesticides standard mixture investigated, with OV-1701, IL60 and IL111 columns, while Table 2 reports the chromatographic performance of IL columns compared to OV-1701, again taken as reference because of its efficiency and inertness. Again, IL76 was discarded because its performance was not comparable to those of the other investigated IL phases.

These results show that, with low volatility compounds, IL column average peak widths are all very close or equal to those of OV-1701, while average tailing factors all fall within a relatively narrow range, in particular compared to those of the highly volatile suspected allergens discussed above. With high boiling compounds, again the performance of IL60 was equal to that of OV-1701, but the other ILs also gave results comparable to that of OV-1701, including the highly polar IL100 and IL111; the maximum values for average peak width (σ: 2.34 s) and tailing factor (1.17) were given by IL82.

Similar considerations can be made for adsorption, where IL columns were in general inert with the investigated pesticides, with the exception of chlorpyriphos (2) (significantly adsorbed on all IL columns) and metalaxyl (6) (partially adsorbed), as is also evident from the chromatograms in Figure 3. Conversely, the good “aptitude” of IL columns for high boiling compounds, and in particular for the investigated pesticides, was confirmed by their separation capability, here represented by the separation measure Δs. In all cases it was better than that of OV-1701 (up to about 50%) and increased with IL polarity (Δs: 966 for IL111), meaning that these columns are of considerable interest for medium-to-high polarity compounds eluting at temperatures above 150°C. IL60 was confirmed as the column giving the best chromatographic performance also for low-volatility compounds.

3.3 Analysis of essential oils of different complexity

IL column performance was then evaluated with the analysis of two essential oils of different chemical composition and complexity, i.e. cornmint and vetiver essential oils. These applications mainly aim to focus on IL column selectivity, which is fundamental in the essential oil and fragrance

fields, because essential oils very often consist of isomers and analogues having very similar mass spectra, making chromatographic data on two columns with different selectivity indispensable for component identification [21,24]. GC linear retention indices (I^Ts) therefore play a fundamental role in this field. Additional experiments were carried out to establish the most effective reference set between homologous series of hydrocarbons and fatty acid methyl or ethyl esters, in terms of I^Ts repeatability and intermediate precision. As expected, the best results were obtained with fatty acid methyl or ethyl esters as reference set, because they are more compatible with the polarity of the IL stationary phases (data not reported).

From the above results, and in consideration of the polarity of the sample components, IL59, IL82, IL61 and IL60 were chosen to be compared to conventional columns (SE-52, OV-1701 and PEG).

3.3.1. Cornmint essential oil

This essential oil has relatively low complexity and is mainly characterized by monoterpenoids and minor amounts of sesquiterpene hydrocarbons; the characterizing groups of peaks to be separated are oxygenated *p*-menthane derivatives, e.g. menthol isomers and esters, and isopulegol, whose abundance is important to discriminate peppermint from cornmint essential oil, and detect adulterations due to the cheaper price of cornmint essential oil [25]. PEG is the conventional stationary phase usually adopted for mint essential oil analysis. Figure 4 shows the GC patterns of cornmint essential oil with the seven columns investigated, and Table 3 the average σ, Δs and tailing factor. This figure clarifies the different selectivity of IL columns compared to conventional columns.

IL60 was again the IL column showing chromatographic efficiency closest to that of conventional OV-1701 and PEG; its average peak width was slightly higher than OV-1701, while its average tailing factor and Δs were similar. However, the column with the best selectivity for this essential oil was IL61, which provided base-line separation of all characterizing components (including menthol isomers and isopulegol) and at the same time showed the lowest number of peaks (6) with adsorption above 10% compared to OV-1701. This essential oil was also used to evaluate absolute peak area repeatability and intermediate precision of IL columns. The same cornmint sample was analysed six times over one day, and three times every two weeks for three months, on IL61 and IL60 columns; the repeatability (RSD% values) of the absolute areas of 22 components were determined. The results were highly satisfactory, since RSD% ranged from 0.2% for α-pinene to 1.9% for neoisomenthol with IL 61, and from 0.6% for isopulegol to 2.3% for β-myrcene for IL 60. Similar results were obtained with intermediate precision, for which RSD% was between 1.5% for α-pinene and 3.9% for neoisomenthol with IL61 and from 1.4% for isopulegol to 3.6 % for β-myrcene for IL60. These results confirm that IL columns are also reliable for quantitative analyses; in spite of their relatively higher adsorption compared to PEG (the stationary phase usually adopted for mint essential oil analysis) they presented consistent performance over time, as was confirmed with the true quantitation of isopulegol in cornmint and peppermint

essential oils [26], an analysis typically used to detect any peppermint essential oils adulteration (data not reported). The results of these experiments on cornmint essential oil were also used to evaluate retention time stability over time: for all compounds considered, over the period investigated, RSD% never exceeded 0.8%.

3.3.2 Vetiver essential oil

Vetiver essential oil is a highly complex essential oil, mainly consisting of sesquiterpenoids, the main fraction being hydrocarbons (mainly m.w. 204) and alcohols (m.w. 220 and 222) and to a lesser extent di-alcohols, ketones (mainly m.w. 218 -220) and acids. This essential oil is widely used in the perfume industry, and its composition was studied in detail by Weyestahl et al. [22], who characterized and identified 144 components. Different chemotypes and qualities, with different commercial values mainly depending on their origins, are available commercially. However, the estimated number of components is around 200. In this study, the essential oil was fractionated into two main fractions, separating hydrocarbons from oxygenated fractions, by column chromatography on silica gel to facilitate evaluation of the selectivity performance of IL stationary phases.

Analysis of vetiver essential oil illustrates the true potential of IL columns, in terms of both selectivity and discrimination between chemical classes. Figure 5 shows the GC profiles of the essential oil investigated, analysed on the seven columns considered, while Figure 6 reports the IL60 GC patterns of the total essential oil and of the hydrocarbon and oxygenated fractions. Table 4 gives average peak width, separation measure, and number of separated peaks, for the total oil and for the hydrocarbon and oxygenated fractions.

These analyses relate the selectivity of IL columns to the chemical groups of essential oil components: in particular, IL59 and IL60 separate hydrocarbon, alcohol, ketone, acid and di-alcohol groups very effectively in the chromatogram. This separation has been confirmed by the GC patterns and component mass spectra of the essential oil fractions. The three conventional columns (PEG included) were unable to give such a clear distinction. This result is very useful because, in essential oil quality control in general and in that of this essential oil in particular, the ratio between total abundance of components, subdivided by functional group, is often required to characterize composition.

The above results are also confirmed by values achieved for efficiency, determined on the total oil and on the two fractions. IL60 presented the lowest total average peak width (σ: 2.10 s) of all the columns investigated, and was close to the minimum when the hydrocarbons (σ: 1.98 s) and oxygenated (σ: 2.34 s) fractions were analysed separately. The separation capability was again evaluated through the separation measure; the Δs values of IL59 and IL60 were about three times that of SE-52 and about double that of PEG, most probably because peak coelution with conventional columns contributed to peak width increase, and, as a consequence, negatively influenced Δs. These experiments also emphasize the major advantage of IL columns in the

analysis of this essential oil, i.e. the number of peaks separated, which is by far higher than is offered by conventional columns: 81 and 84 for SE-52 and PEG, versus 127 for IL60. The results for hydrocarbons and oxygenated fractions were even better: IL60 separated 91 and 68 components, respectively. The importance of these results is evident, considering that almost all these hydrocarbons are isomers belonging to the sesquiterpene group (i.e. $C_{15}H_{24}$, m.w.: 204) with very similar and non-diagnostic mass spectra. Their identification is therefore only possible through their retention indices, meaning that the best possible separation is necessary for reliable identification. The same considerations can be made for the sesquiterpenic alcohols (i.e. $C_{15}H_{24}O$, m.w.: 222).

4. CONCLUSIONS

The results show that ILs can now successfully be used as GC stationary phases for routine analysis, thanks to the combination of their peculiar selectivity and the continual improvement in their chromatographic properties. The examples reported here show that IL columns can be used for a wide range of applications characteristic of the flavour, fragrance and essential oil fields, mainly because of their non-common selectivity, in particular when a separation on functional groups is required. IL column performance is now comparable to, or only slightly lower than, those of the conventional columns routinely used in this fields. The figures of merit of the columns investigated here, measured on the tested samples, confirm that their chromatographic properties have drastically improved with their evolution over time, as is also shown by the continual increase in their efficiency, inertness and separation capability. However, further effort must still be made in column manufacturing to achieve inertness comparable to that of conventional columns, at the same time reducing their activity. Conversely, further studies are needed to improve our understanding of the IL GC-separation mechanism under isothermal and programmed temperature conditions, and to investigate the stability over time of their performance at high temperatures.

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References

[1] C.F. Poole, S.K. Poole, J. Sep. Sci. 34 (2011) 888.

[2] C. Ragonese, D. Sciarrone, P.Q. Tranchida, P. Dugo, L. Mondello, J. Chromatogr. A 1255 (2012) 130.

[3] C. Yao, J.L. Anderson, J. Chromatogr. A 1216 (2009) 1658.

[4] D.W. Armstrong, L.F. He, Y.S. Liu, Anal. Chem. 71 (1999) 3873.

[5] S.C. Dhanesar, M.E. Coddens, C.F. Poole, J. Chromatogr. 349 (1985) 249.

[6] S.C. Dhanesar, C.F. Poole, Anal. Chem. 56 (1984) 2509.
[7] J.L. Anderson, D.W. Armstrong, Anal. Chem. 75 (2003) 4851.
[8] J.L. Anderson, D.W. Armstrong, Anal. Chem. 77 (2005) 6453.
[9] D.W. Armstrong, T. Payagala, L.M. Sidisky, LC GC Eur. 22 (2009) 459.
[10] K. Huang, X. Han, X. Zhang, D.W. Armstrong, Anal. Bioanal. Chem. 389 (2007) 2265.
[11] T. Payagala, Y. Zhang, E. Wanigasekara, K. Huang, Z.S. Breitbach, P.S. Sharma, L.M. Sidisky, D.W. Armstrong Anal. Chem. 81 (2009) 160.
[12] G. Purcaro, P.Q. Tranchida, C. Ragonese, L. Conte, P. Dugo, G. Dugo, L. Mondello, Anal. Chem. 82 (2010) 8583.
[13] C. Ragonese, D. Sciarrone, P.Q. Tranchida, P. Dugo, G. Dugo, L. Mondello, Anal. Chem. 83 (2011) 7947.
[14] M. Qi, D.W. Armstrong, Anal. Bioanal. Chem. 388 (2007) 889
[15] C. Ragonese, P. Donato, D. Sciarrone, P. Dugo, G. Dugo, L. Mondello, in T. Sandra, P. Sandra (Editors), 32th ISCC & 5th GCxGC Symposium, Riva del Garda, 26-30 May, 2008, p. 130.
[16] C. Ragonese, P.Q. Tranchida, P. Dugo, G. Dugo, L.M. Sidisky, M.V. Robillard, L. Mondello, Anal. Chem. 81 (2009) 5561.
[17] Q. Gu, F. David, F. Lynen, P. Vanormelingen, W. Vyverman, K. Rumpel, G.W. Xu, P. Sandra, J. Chromatogr. A 1218 (2011) 3056.
[18] P. Delmonte, A.R.F. Kia, J.K.G. Kramer, M.M. Mossoba, L. Sidisky, J.I. Rader, J. Chromatogr. A 1218 (2011) 545.
[19] C. Reyes-Contreras, C. Domìnguez, J.M. Bayona, J. Chromatogr. A (2012)http://dx.doi.org/10.1016/j.chroma.2012.05.082.
[20] DIRECTIVE 2003/15/EC of the European Parliament and of the Council of 27 February 2003.
[21] B.D. Zellner, C. Bicchi, P. Dugo, P. Rubiolo, G. Dugo, L. Mondello, Flavour Frag. J. 23 (2008) 297.
[22] P. Weyerstahl, H. Marschall, U. Splittgerber, D. Wolf, H. Surburg, Flavour Frag. J. 15 (2000) 395.
[23] L.M. Blumberg, J. Chromatogr. A 1218 (2011) 5375.
[24] P. Rubiolo, B. Sgorbini, E. Liberto, C. Cordero, C. Bicchi, in A. Herrmann (Editor), In The Chemistry and biology of volatiles, Wiley, 2010, p. 39.
[25] J.S Spencer., E.Dowd, W.Faas, Perfumer & Flavorist 22 (1997) 37.
[26] C. Bicchi, E. Liberto, M. Matteodo, B. Sgorbini, L. Mondello, B.D. Zellner, R. Costa, P. Rubiolo, Flavour Frag. J. 23 (2008) 382.

Figure 1 GC-FID patterns of the allergen standard mixture with the columns investigated. For peak identification see paragraph 2.1 (peak numbers follow elution order on IL60 column), and for chromatographic conditions and column acronyms, see paragraph 2.2.

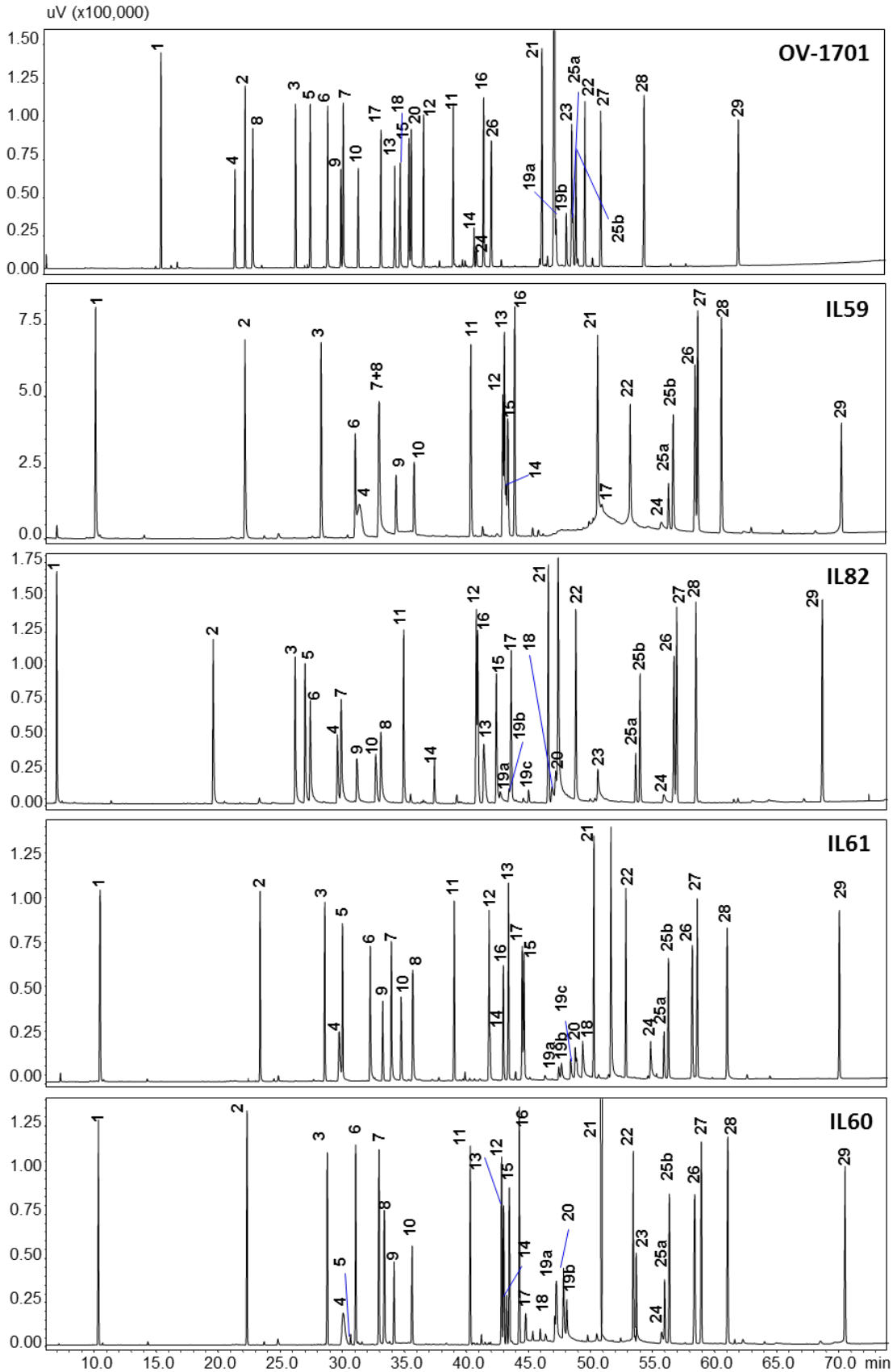

Figure 2 Adsorption of suspected allergens, calculated from the absolute area ratio of each compound with each investigated IL *versus* OV-1701 columns, taken as reference.

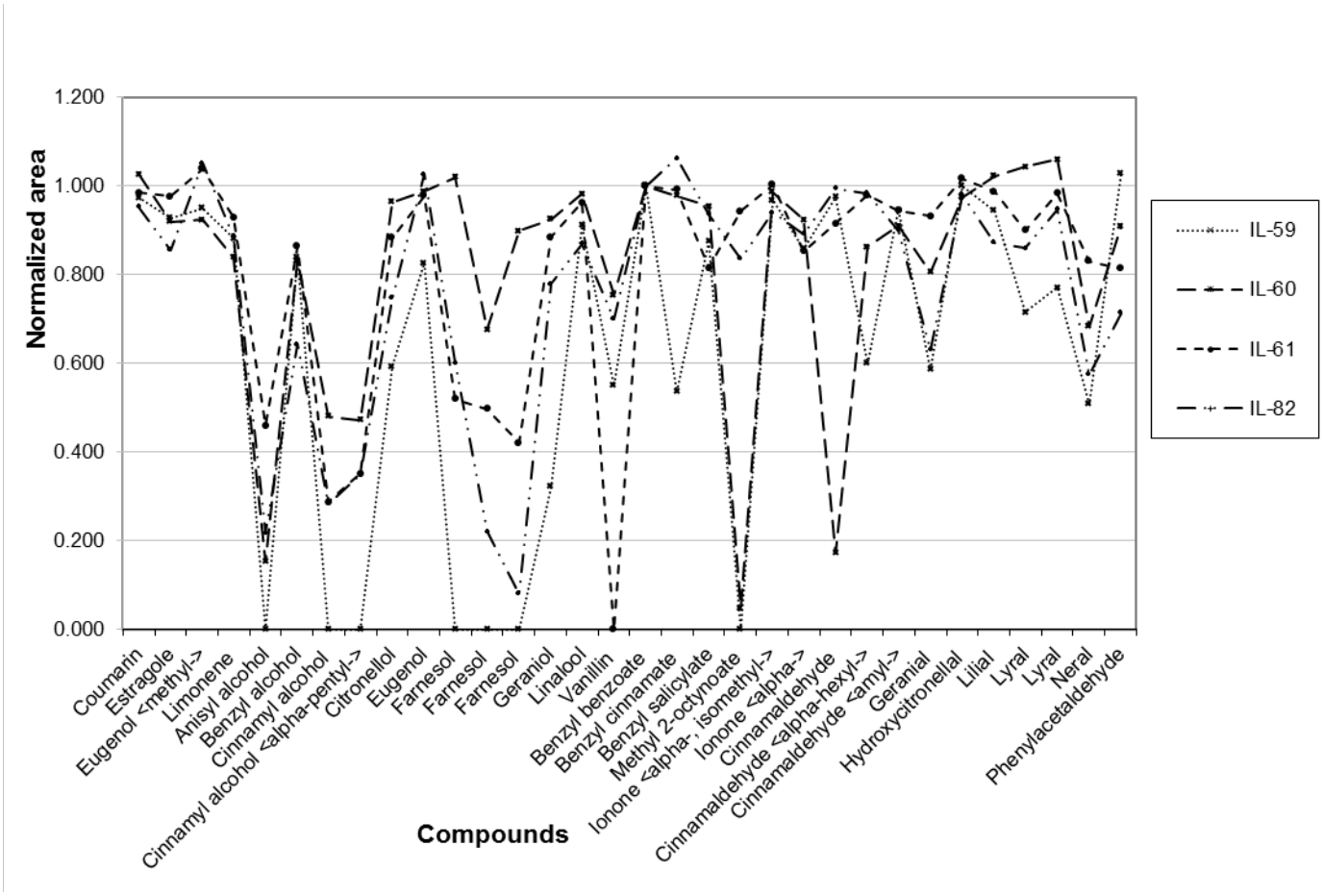

Figure 3 GC-FID patterns of the pesticide standard mixture with OV-1701, IL111 and IL60 columns. For peak identification see paragraph 2.1 (peak numbers follow elution order on IL60 column), and for chromatographic conditions and column acronyms, see paragraph 2.2.

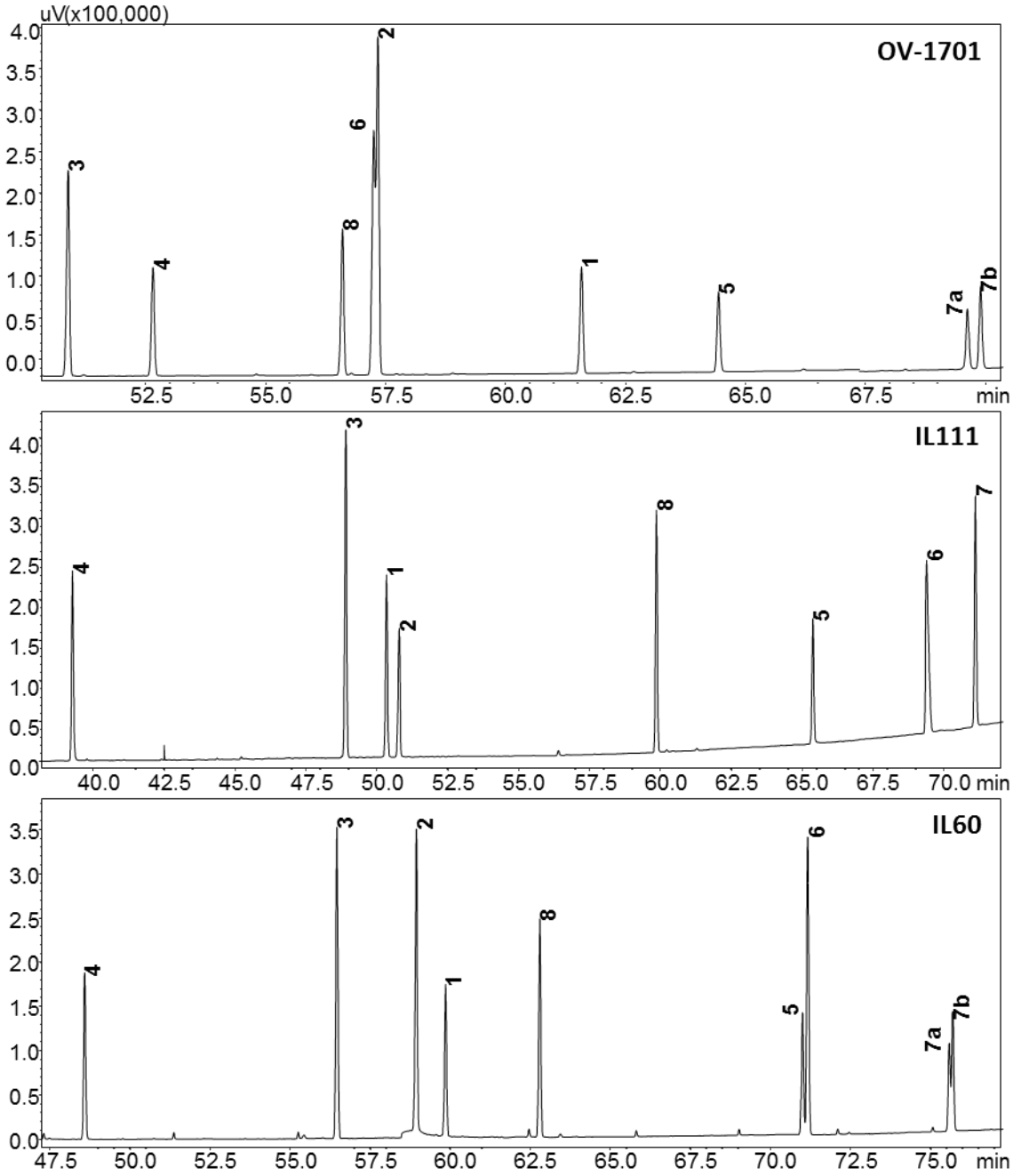

Figure 4 GC-FID patterns of cornmint essential oil with the seven columns investigated. For chromatographic conditions and column acronyms, see paragraph 2.2. Peak identification (peak numbers follow elution order on IL60 column): 1: α-pinene, 2: β-pinene, 3, β-myrcene, 4: limonene, 5: 1,8-cineole, 6: 3-octanol, 7: linalool, 8: β-bourbonene, 9: neomenthol, 10: isovalerate 3-hexenyl, 11: isopulegol, 12: neoisomenthol, 13: *t*-β-caryophyllene, 14: menthol, 15: menthone, 16: α-terpineol, 17: menthyl acetate, 18: isomenthone, 19: germacrene D, 20: isopulegone, 21: pulegone, 22: piperitone.

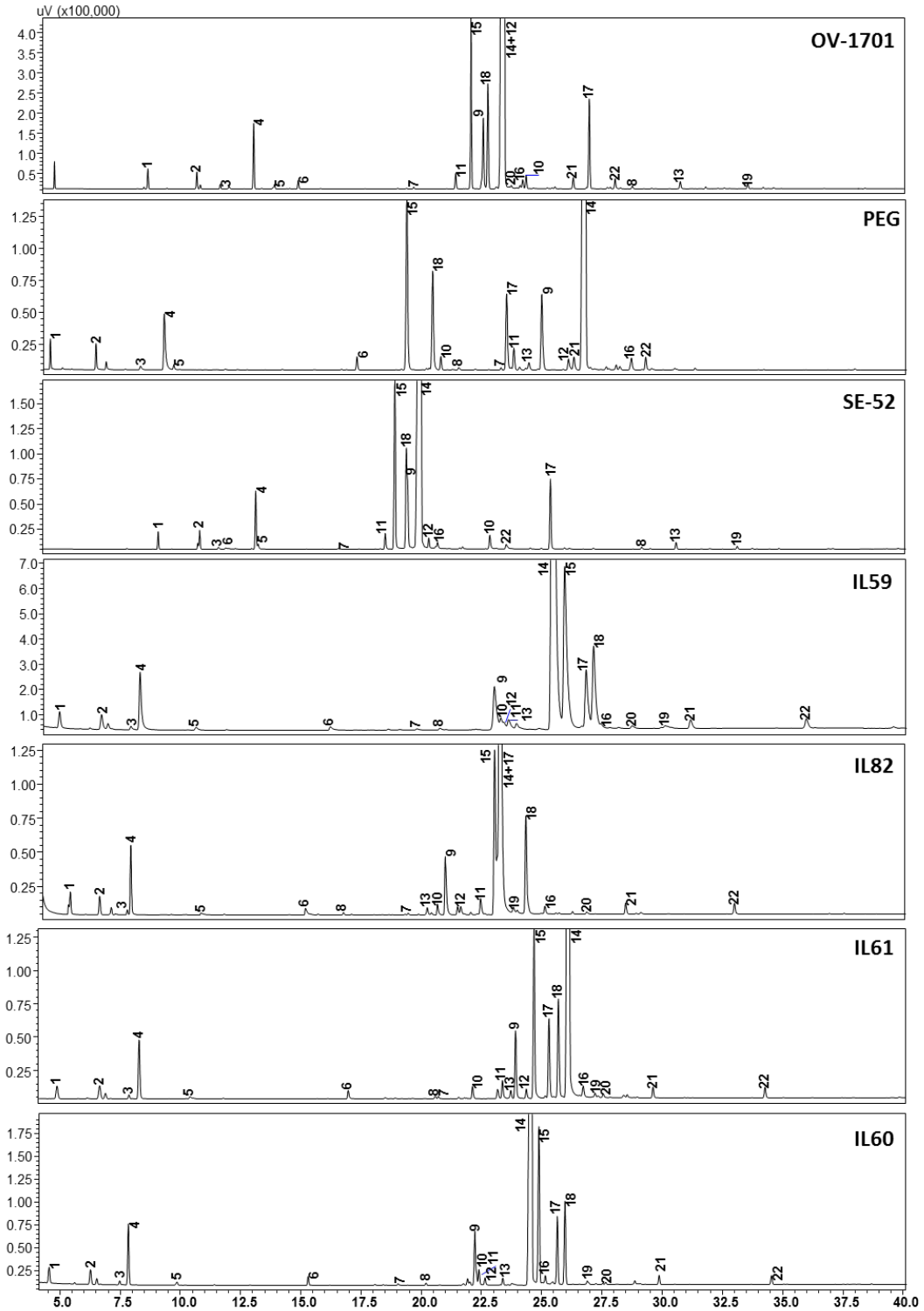

Figure 5 GC-MS patterns of vetiver essential oil with the seven columns investigated. For chromatographic conditions and column acronyms, see paragraph 2.2. Legend: hydrocarbons, alcohols, ketones, dialcohols, acids.

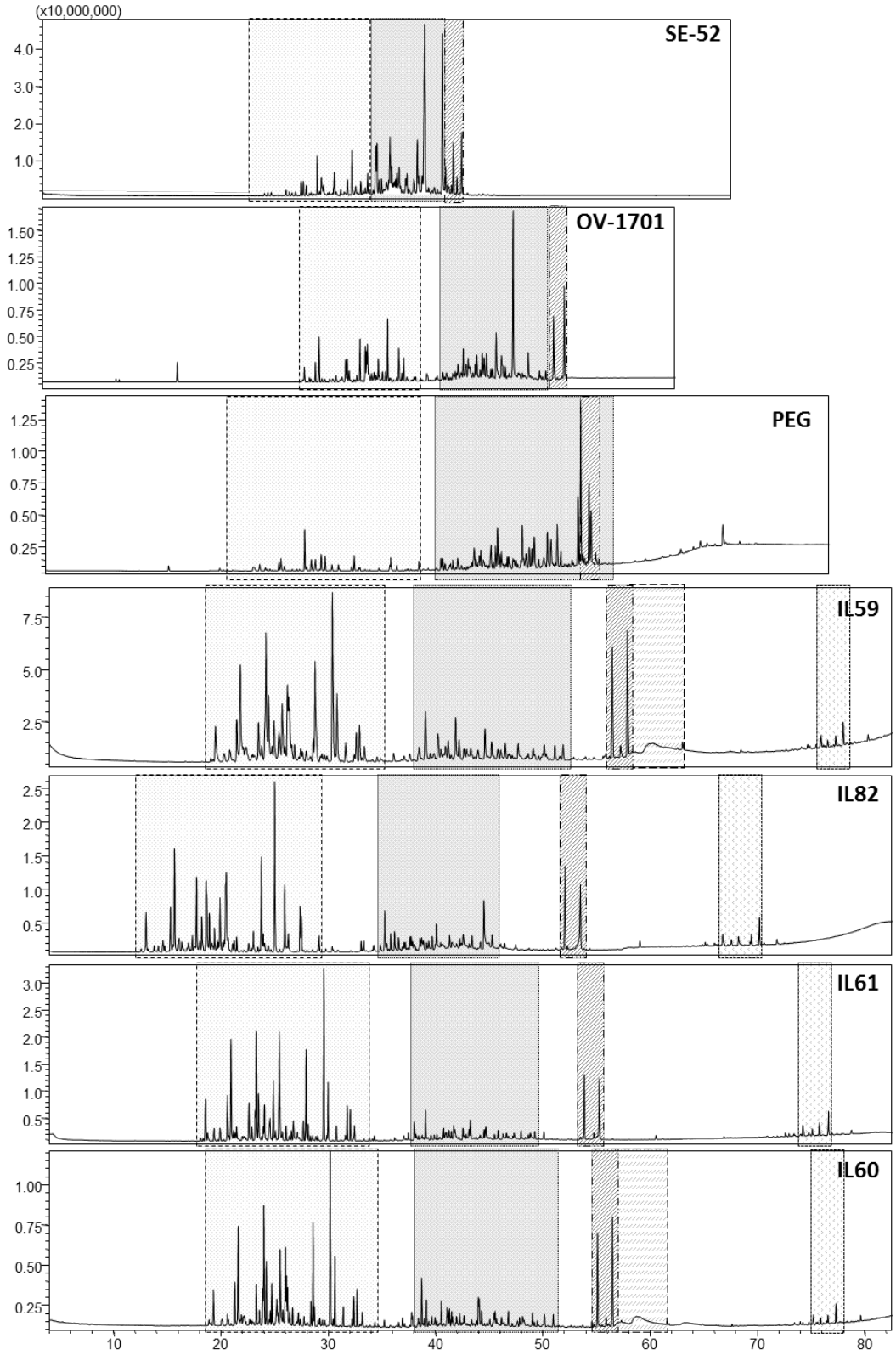

Figure 6 GC-MS patterns of total vetiver essential oil (a) and its hydrocarbon (b) and oxygenated (c) fractions, analyzed with the IL60 column. For chromatographic conditions and column acronyms, see paragraph 2.2.

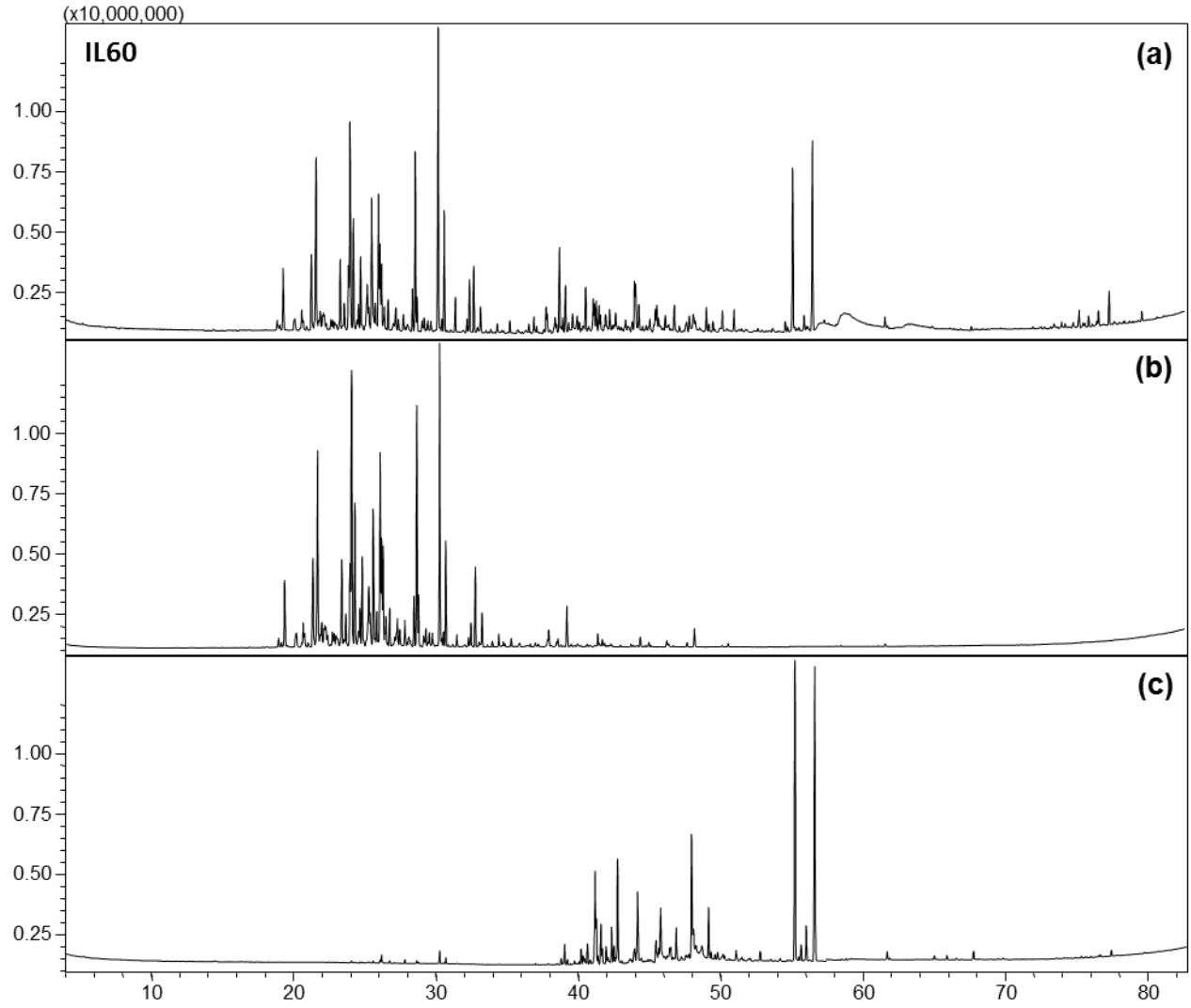

Table 1: average peak width (σ), average tailing factor, number (#) of asymmetric peaks, number (#) of adsorbed peaks and separation measure (*Δs*) for the allergen standard mixture with the columns investigated.

	Average σ	Average tailing factor	# asymmetric peaks	# adsorbed peaks	*Δs*
OV-1701	0.031	0.931	2		1582
SE-52	0.036	1.074	8	7	1121
PEG	0.038	1.214	11	7	1363
IL59	0.062	1.214	32	21	1516
IL82	0.049	1.537	25	20	1787
IL61	0.038	1.217	17	15	1791
IL60	0.038	0.993	10	13	1744

Table 2: average peak width (σ), average tailing factor, number (#) of adsorbed peaks and separation measure (*Δs*) for the pesticides standard mixture with the columns investigated.

	Average σ	Average tailing factor	# adsorbed peaks	*Δs*
OV-1701	0.031	0.969		625
IL59	0.038	1.103	3	698
IL82	0.039	1.170	2	775
IL100	0.039	0.866	3	835
IL111	0.035	1.108	2	966
IL61	0.035	1.110	2	713
IL60	0.031	0.974	3	847

Table 3: average peak width (σ), average tailing factor and separation measure (*Δs*) for cornmint e.o. with the columns investigated.

	Average σ	Average tailing factor	*Δs*
OV-1701	0.024	1.020	1049
PEG	0.025	1.193	993
SE-52	0.031	1.147	969
IL59	0.054	1.675	580
IL82	0.034	1.569	873
IL61	0.037	1.111	803
IL60	0.030	0.958	988

Table 4: average peak width (σ), separation measure (*Δs*) and number (#) of separated peaks for the total vetiver e.o. and for the hydrocarbon and oxygenated fractions with the columns investigated.

	Average σ			***Δs***			**# of separated peaks**		
	Total e.o.	Hydrocarbon fraction.	Oxygenated fraction	Total e.o.	Hydrocarbon fraction	Oxygenated fraction	Total e.o.	Hydrocarbon fraction	Oxygenated fraction
SE-52	0.042	0.043	0.047	584	283	208	84	73	51
OV-1701	0.038	0.033	0.040	760	304	329	94	68	51
PEG	0.037	0.036	0.038	965	503	411	81	65	57
IL59	0.037	0.035	0.036	1804	464	636	97	70	48
IL82	0.045	0.040	0.062	1555	445	430	64	46	38
IL61	0.044	0.038	0.054	1264	383	528	109	72	60
IL60	0.035	0.033	0.039	1882	422	587	127	91	68