IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils: Quantitative GC–MS analysis of officially-regulated allergens in perfumes

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1718193 since 2020-01-14T12:59:47Z

Published version:

DOI:10.1016/j.chroma.2019.460567

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

29 May 2023

Ionic liquids as water-compatible GC stationary phases for the analysis of fragrances and essential oils: quantitative GC-MS analysis of officially-regulated allergens in perfumes

Maria Mazzucotelli[1$], Manuel A. Minteguiaga[1,3$], Barbara Sgorbini[1], Len Sidisky[2], Arianna Marengo[1], Patrizia Rubiolo[1], Carlo Bicchi[1], Cecilia Cagliero[1*]

[1] Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, I-10125 Torino, Italy

[2] MilliporeSigma 595 N. Harrison Rd.| Bellefonte, PA, 16823, USA

[3] Laboratorio de Biotecnología de Aromas, Departamento de Química Orgánica, Facultad de Química, Universidad de la República, General Flores 2124, Montevideo, 11800, Uruguay

*Corresponding author
Cecilia Cagliero
Dipartimento di Scienza e Tecnologia del Farmaco
Università degli Studi di Torino
Via P. Giuria 9, I-10125 Torino, Italy
e-mail: cecilia.cagliero@unito.it

[$] Maria Mazzucotelli and Manuel A. Minteguiaga gave an equivalent contribution to this study

Abstract

Qualitative and quantitative determination of volatile markers in aqueous based fragrances assumes ever-increasing importance, because of both the need for quality control and the safety-regulatory limitations introduced for several compounds. This study reports and critically discusses the results of applying new water-compatible ionic-liquid (IL) GC stationary phases, based on phosphonium and imidazolium derivative cations combined with trifluoromethanesulphonate (Watercol^{TM}) to the direct quantitative analysis of aqueous samples in the perfume field with GC-MS. Narrow-bore columns of different lengths, especially prepared for this study, were adopted to minimize the amount of water reaching the MS detector after GC separation. All GC-MS analysis steps were investigated, to achieve results compatible with quality control requirements for the volatiles of interest in this field, in terms of LODs, LOQs, and repeatability. Reliability of the GC-MS results was demonstrated by determining volatile allergens in two commercial perfumes, as per EU regulations concerning no-declaration limits for leave-on (0.001%) and rinse-off (0.01%) cosmetic products.

Keywords - Ionic liquids; Water-compatible stationary phases; GC and GC-MS; Aqueous samples; Perfumes; Volatile allergens

1. Introduction

Quali-quantitative determination of volatile markers in aqueous products based on essential oils and, more in general, on fragrances, is increasingly necessary. This is not only due to quality control requirements, but also to regulatory limitations introduced for some compounds. An important example is the no-declaration limits, at 0.01% for leave-on and 0.001% for rinse off products that the EU indicated for 24 volatile allergens in cosmetics, in Directive 2003/15/EC and subsequently in Regulation (EC) No 1223/2009 on cosmetics, following the opinion of the Scientific Committee on Consumer Safety (SCCS) published in 1999 [1-3]. More recently, in 2011, the SCCS wrote to the European Commission with a further opinion, proposing an extended list of "established contact allergens in humans" consisting of 54 chemicals and 28 natural extracts [4]. Although gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) are the techniques of choice for analyzing volatiles, it is well-known that water is poorly compatible with conventional GC stationary phases (SPs), most of which are hydrophobic, and can therefore generate interferences in the direct analysis of aqueous samples. This incompatibility produces phase degradation, peak broadening, asymmetry, and adsorption, resulting in poor sensitivity and efficiency, and thus unsatisfactory limits of detection and, especially, of quantitation [5,6]. Routine target analyte quantitation in water samples thus frequently requires a preliminary sample preparation step to be included in the method that, however, may be time-consuming and/or affect recovery; it often produces discrimination among the components of a sample.

The direct analysis of water as sample component has generally been achieved by using packed columns filled with Porapak, or wall-coated PoraPLOT columns, but these methods are mainly for water as analyte, and in general require further analyses with conventional columns to evaluate other target components in the sample [5]. A possible solution to this problem was proposed by Armstrong et al. who, in 2012, introduced new stationary phases (SPs) based on ionic-liquids (ILs); these showed high stability and compatibility with water as analyte to be determined in a sample. These ILs consisted of phosphonium and imidazolium derivatives as cations, combined with 2 or 3 units of trifluoromethanesulphonate as anions. The same group also reported a number of applications concerning the determination of water as analyte in the pharmaceutical and food fields with the same and other IL derivatives [7-9]. In 2016, a set of columns coated with the above ILs with different retention properties were marketed by MilliporeSigma under the trade name Watercol™ [10].

In a previous article, Cagliero et al. reported the results of a study extending the use of commercially-available water-compatible IL columns for the direct analysis of aqueous samples in the fragrance and essential oil fields, by GC with thermal conductivity (TCD) and/or flame ionization detectors (FID) [11]. In particular the columns investigated were Watercol™ 1460, i.e. coated with tri-(tripropyl-phosphoniumhexanamido)-triethylamine trifluoromethanesulfonate (hereafter 1460), and Watercol™ 1910, i.e. coated with 1,11-di-(3-hydroxyethylimidazolium)-3,6,9-trioxaundecane trifluoromethane sulfonate (hereafter 1910). More

recently, Sgorbini et al. [12] successfully applied Watercol™ in an experimental study comparing different methods, to measure the transfer rate and human intake of volatile bioactive compounds, from herbal teas prepared with medicinal and aromatic plants. These studies showed that water-compatible IL stationary phases were very promising for qualitative and quantitative analysis of target analytes in aqueous or hydroalcoholic samples of fragrances, herbal teas and essential oils. At the same time, the results emphasized that, in view of their routine application in quality control, both the efficiency and inertness, as well as the maximum operative temperature of the investigated IL columns, required further improvement, and that mass spectrometry must be used as GC detector if their use is to be fully compatible with regulatory requirements. The reliability of a MS system as detector for these applications might be affected by the repeated introduction of relatively large amounts of water, due to the large number of samples processed in a quality control laboratory (in particular, in quantitative analysis) since water at high temperature and under high vacuum is highly reactive and abrasive, and can affect the filament, and more in general the performance and stability of the ion source.

The present study aims to combine GC with water-compatible columns (Watercol™) with an electron ionization quadrupole-MS (EI-MS) detector to analyse target analytes in aqueous or hydroalcoholic samples. Column characteristics, injection conditions, and modality of introduction of water (vapor) in the MS detector are discussed critically in-depth, focussing chiefly on quantitative analysis of volatile allergens in fragrances for which regulatory organizations impose declaration limits, and allergen determination in two commercial perfumes. This important quality-control problem for the perfume industry has already been dealt with in official methods from professional organizations and research groups in this field [13-15]; the analysis of volatile allergens has here been taken as a case study for effectively testing Watercol™ reliability for routine applications, because of the widely differing structures of the compounds involved, and the high complexity of aqueous perfume matrices.

2. Material and Methods

2.1 Samples and chemicals

Column performance was evaluated with the Grob Test [16] (Merck, Milan, Italy). Stock solutions of the 24 volatile allergens and 5 other related compounds, at 5000 mg/L in cyclohexane, and in a 1:1 ethanol/water mixture (hereafter EtOH/H_2O), were prepared from pure standards (all from Merck, Milan, Italy). Deionized water was obtained from a Milli-Q purification system, Millipore, Merck, Milan, Italy). The mixtures of volatile allergens and related compounds included: **1.** benzyl alcohol (CAS: 100-51-6), **2.** phenylacetaldehyde (CAS: 122-78-1), **3.** cinnamaldehyde (CAS: 104-55-2), **4.** cinnamyl alcohol (CAS: 104-54-1), **5.** limonene (CAS: 138-86-3), **6.** anisyl alcohol (CAS: 105-13-5), **7.** coumarin (CAS: 91-64-5), **8.** estragole (CAS: 140-67-0), **9.** vanillin (CAS: 121-33-5), **10.** geranial (CAS: 5392-40-5), **11.** neral (CAS: 106-26-3), **12.** methyl-2-octynoate (CAS: 111-12-6), **13.** geraniol (CAS: 106-24-1), **14.** linalool (CAS: 78-70-6), **15.** β-citronellol (CAS: 106-22-9), **16.** eugenol

(CAS: 97-53-0), **17.** hydroxycitronellal (CAS: 107-75-5); **18.** methyl eugenol (CAS: 93-15-2), **19.** α-ionone (CAS: 127-41-3), **20.** amyl cinnamaldehyde (CAS: 122-40-7), **21.** lilial (CAS: 80-54-6), **22.** α-pentyl cinnamyl alcohol (CAS 101-85-9), **23.** α-isomethyl ionone (CAS: 127-51-5), **24a.** lyral isomer a (CAS: 51414-25-6) **24b.** lyral isomer b (CAS: 31906-04-4), **25.** benzyl benzoate (CAS: 120-51-4), **26.** hexyl cinnamaldehyde (CAS: 101-86-0), **27.** farnesol isomers (CAS: 106-28-5), **28.** benzyl salicylate (CAS: 118-58-1) and **29.** benzyl cinnamate (CAS: 103-41-3). The working solutions (30 mg/L for each analyte) were prepared by suitable dilution of the stock solution in the same solvents and directly injected into the GC systems.

Two commercial perfumes labelled as "eau de toilette" (perfume 1 and perfume 2) were obtained from a local supermarket and injected into the GC and GC-MS systems after dilution at 1:10 or 1:20 in EtOH/H_2O solution. Standard mixtures of the following compounds were prepared for the quantitation of allergens in these perfumes: **1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.** linalool, **15.** β-citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** lilial, **23.** α-isomethyl ionone, **24a.** and **24b.** lyral isomers, **26.** hexyl cinnamaldehyde and **30**. linalyl acetate (CAS: 115-95-7). Linalyl acetate was also included because it is comprised in the extended EU list of 54 compounds to be monitored [14]. Carvacrol (CAS: 499-75-29) was provided by Merck and was used as internal standard (ISTD) for quantitation because it does not co-elute with any other perfume component under the analysis conditions applied.

2.2 Analysis conditions

2.2.1 Instrumental set-up

A Shimadzu 2010 GC-FID-TCD system equipped with an autosampler AOC-20i and combined with GC Solution Version 2.30.00 SU6 software (Shimadzu Co., Kyoto, Japan) was adopted to optimize injection conditions for aqueous samples. The two detectors were used separately. GC-MS analyses were carried out on an Agilent 6890N GC coupled to a 5975 MSD provided with a 7683B autosampler and a ChemStation Version E.02.02.1431 data processing system (Agilent Technologies, Santa Clara, CA).

2.2.2 Columns

Four non-bonded narrow-bore (NB) capillary Watercol™ columns from MilliporeSigma (Bellefonte, PA, USA) were used: two 1460 [Tri-(tripropylphosphoniumhexanamido)-triethylamine-trifluoromethane sulfonate; T_{max}: 260°C] and two 1910 [1,11-Di-(3-hydroxyethylimidazolium)-3,6,9-trioxaundecane trifluoro methane sulfonate; T_{max}: 180°C]. Column characteristics: d_c: 0.10 mm; d_f: 0.08 µm; length: 10 and 15 m. The investigated NB columns were specifically prepared for this study, and submitted to a dedicated property deactivation procedure**.** The two types of column are characterized by different operative temperatures: 1460 operates from 60°C to 260°C, and 1910 between 40°C and 180°C. A NB OV-1701 column (length: 10 m, d_c: 0.10 mm; d_f: 0.10 µm) was used as reference.

2.2.3. Optimization of injection conditions for aqueous samples

Injection conditions were optimized on GC-FID with 15 m capillary columns, on the standard mixture of the 29 allergens and related compounds (hereafter allergens) with hydrogen as carrier gas. The investigated conditions were: i) liners without or with 0.5 or 1cm glass wool plug, and internal volume of about 800 µL for GC-FID-TCD (Agilent Crosslab P/N: 8001-0104); ii) injection pressure: 200 kPa or overpressurized from 250 to 450 kPa; and iii) split ratios: 50, 70 and 100. The optimized conditions were then adapted to GC-MS equipped with 10 m columns and helium as carrier gas, and with a liner with internal volume about 900 µL (Agilent P/N: 5190-2293). GC analyses were carried out with syringes for injection provided with 5.1 mm needles and liners filled with 1 cm glass wool plug, thus assuring for both GC-FID and GC-MS systems, a distance of at least 3 cm between the needle tip and the glass wool plug.

2.2.4. GC-FID-TCD conditions

GC-FID-TCD analyses (with 15 m columns) were carried out under the following conditions: oven temperature program 40°C (2 min) // 3°C/min // 200°C (5 min) for 1460; 40°C (2 min) // 3°C/min // 180°C (5 min) for 1910. Injector temperature: 230°C for 1460; 200°C for 1910, detector temperature (FID or TCD): 230°C. Sampling rate for both detectors: 40 msec. Carrier gas: H_2 (FID), He (TCD); column flow: 0.4 mL/min in both cases; initial head pressure: 199 kPa for H_2 and 332 kPa for He. Split ratio: 1:100 (if not specified otherwise). TCD current: 60 mA, signal polarity: positive. TCD make-up flow: 8.0 mL/min.

2.2.5. GC-MS Conditions

GC-MS analyses (with 10 m columns) were carried out under the following conditions: oven temperature: 40°C (2 min)//3°C/min//200°C (5 min) for 1460; 40°C (2 min)//3°C/min//180°C (5 min) for 1910. Injector temperature: 230°C for 1460; 200°C for 1910. MS interface temperature: 230°C in both cases. The 1910 column was connected to the MS through a post-column of deactivated fused silica (0.5 m x 0.10 mm d_c) (Mega, Legnano, Italy) to make it compatible with the higher interface temperature. Injection mode: split; ratio: 1:100; injection volume: 1.0 µL. Carrier gas: He; column flow: 0.4 mL/min. Initial head pressure (He): 240 kPa. An overpressure injection (pulsed split, He) of 350 kPa for 1.0 min was applied for the aqueous samples. Ion source and analyzer (quadrupole) temperatures: 230°C and 150°C, respectively. Ionization mode: electron impact at 70 eV; acquisition mode: scan (35-350 m/z). Solvent delay: 3.0 min for samples in cyclohexane. For aqueous samples, a solution 1/10 of the perfume 1 in EtOH/H_2O was injected once into each Watercol™ column, with MS scan range set between 17 and 350 m/z, to establish the elution time interval of the H_2O peak. This value was then used to set the time point at which to switch off the ion-source filament (as solvent delay) in routine quantitative analyses, resulting in a solvent delay of 5.00 min for 1460, and 7.40 min for 1910.

2.2.6. Analyte identification and quantitation

The components of the commercial perfumes were identified by comparison of their mass spectra with those of authentic standards, or stored in commercial or in-house libraries. The results were confirmed by those of a previous publication of the authors' on commercial perfumes [11].

The volatile allergens identified in the perfumes were quantified by building up a calibration curve for each of the 14 target components (**1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.** linalool, **15.** β-citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** lilial, **23.** α-isomethyl ionone, **24a.** lyral isomers a, **24b.** lyral isomers b, **26.** hexyl cinnamaldehyde and **30**. linalyl acetate), by injecting a set of analyte standard solutions, containing 5, 10, 50, 100, 200, 500 and 1000 mg/L of each compound diluted in EtOH/H_2O. Stock solutions of each analyte were therefore prepared at 10.0 mg/mL in 95% EtOH and suitably diluted to the required levels for calibration. A stock solution of carvacrol (ISTD) at 100 mg/mL in EtOH/H_2O was used to evaluate the instrumental repeatability, adding 5.0 µL of it to all samples to achieve a final concentration of 50 mg/L.

The analytes investigated were quantified through the area of one target ion, obtained in extract ion mode (EIM) from the TIC-GC-MS data; two qualifiers, again obtained in EIM, were used for identification. Target ion and qualifiers, selected from among the diagnostic fragments of each analyte, are reported in Table 1. The method linearity was determined within the above concentration range, providing a determination coefficient R^2 above 0.99. Limit of detection (LOD) and limit of quantitation (LOQ) of the method for each analyte were obtained by injecting the quantitation standard mixture at 1, 5, and 10 mg/L solubilized in EtOH/H_2O. LOD was calculated from the average "peak to peak" noise values sampled in the region of elution of each analyte in the chromatogram, with a coverage factor of 3. LOQ was the lowest concentration for which instrumental response integration reported an RSD%, across replicate analyses, below 20%. The repeatability of the system was determined by 6 consecutive injections of a solution of all analytes at 50 mg/L, under the above conditions. Intermediate precision was determined by injecting the same solutions every 3 weeks over a period of 3 months.

3. Results and discussion

Routine quantitative GC-MS analysis of target compounds in aqueous samples by direct injection entails re-evaluating and tuning the three main steps of the analytical procedure, since water as solvent can i) affect column performance, ii) interfere with correct injection and iii) interfere with a reliable MS response. The analytical procedure has therefore been adapted to aqueous samples to obtain reliable, linear, and repeatable results. Analyses were carried out in 1:1 EtOH/H_2O solution chosen as a ratio of compromise due to the widely variable composition of the products of the fragrance field, that range from a highly predominant percentage of ethanol in perfumes (eau de parfum, eau de toilette etc) to high percentage of water in the perfumed waters.

From this standpoint, narrow bore (NB) columns of different lengths, especially prepared for this study, were used because, thanks to their lower loadability, the amount of water introduced into the GC-MS system and, in particular, into the mass spectrometer is minimized and, simultaneously, analysis time is significantly reduced.

This section consists of two main parts: i) the first addresses tuning the optimal GC-MS conditions to obtain reliable results; and ii) the second reports the results obtained on applying the optimized conditions to quantifying volatile allergens in two commercial perfumes.

3.1 Evaluation of column performances and optimization of GC-MS conditions

3.1.1 Watercol™ column performance

The performance stability to injection of aqueous samples, of columns coated with the two IL SPs investigated, was already shown to be very high in previous work [11], by monitoring the consistency of water retention indices after repeated injection (60) of EtOH/H_2O. These results were here confirmed, by analyzing the above mixture consecutively for 15 times with the narrow-bore columns object of this study, by GC-TCD. Water and ethanol retention times were confirmed to be very stable and repeatable (data not shown). The standard mixture of 29 compounds of interest here, 24 of them included on the EU list [2], solubilized in cyclohexane and in 1/1 EtOH/H_2O, was analyzed with both 1460 and 1910 columns, to evaluate column performance. The consistency of analyte retention times, independently of the main solvent applied (cyclohexane or EtOH/H_2O), offers a further indication of column stability (Table 2). Figure 1 reports the GC-FID patterns of the allergen standard mixture, analyzed with Watercol™ 1460 and 1910. Table 2a and 2b reports tailing factors and σ values of the peak width of each analyte (where the peak width at the base line is 4 σ) [17], after injection of standard solutions in cyclohexane and EtOH/H_2O into the two columns, while Figure 2 shows recovery, measured as the ratio of the area of each analyte of the standard mixture in the two solvents analyzed with the two columns, *versus* those obtained with OV-1701. As a preliminary consideration, the results indicate that efficiency and inertness of both columns are very satisfactory, while component recovery shows that the two SPs adsorb some components.

Watercol™ 1460 – Table 2a reports the tailing factors (TF) and σ values of the components of the standard mixture analyzed with this column. Cinnamyl alcohol (**4**), *p*-anisyl alcohol (**6**), vanillin (**9**), and α-pentyl cinnamyl alcohol (**22**) were not detected, probably either because of irreversible adsorption, and/or of retention outside the analysis time range (60 min), while phenylacetaldehyde (**2**) coeluted with neral (**11**), and limonene (**5**), when injected in EtOH/H_2O, coeluted with water.

The 1460 performance with the standard mixture in cyclohexane was good. Most compounds presented tailing factors between 0.9 and 1.1, with the sole exceptions of benzyl alcohol (**1**) (TF 1.2) and lyral 1 (**24a**) and 2 (**24b**), which were 2.0 and 2.2 respectively. The corresponding σ values ranged from 0.021 min for

hydroxycitronellal (**17**) to 0.051 min for linalool (**14**). The high σ value (0.133 min) of limonene (**5**) was probably due to its very low polarity, poorly compatible with the investigated SPs, together with its elution within the solvent queue. Similar (and in some cases better) results were obtained with the same standard mixture in EtOH/H_2O. Here, too, most peaks showed a tailing factor ranging between 0.9 and 1.1, but many were perfectly symmetrical (TF 1.0), unlike the corresponding results with cyclohexane. The only exceptions were again benzyl alcohol (**1**) (TF 1.2) and lyral a (**24a**) and b (**24b**), although their TF values fell to 1.2 and 1.3, respectively. Peak widths were in general in line with those of cyclohexane (sometimes slightly better) and σ values ranged from 0.021 min with hydroxycitronellal (**17**) to 0.051 min for linalool (**14**).

Lastly, recovery percentage vs. OV-1701 was measured using benzyl benzoate (**25**) as internal reference for normalization, its peaks with both 1460 and 1910 being of comparable intensity, as well as of similar symmetry and width. Very few components gave a recovery below 65% with the standard mixture in cyclohexane; those that did were hydroxycitronellal (**17**), eugenol (**16**), and farnesol isomers (**27**), whose recoveries were 55%, 42% and 56% respectively (Figure 2a). The case of the sample in EtOH/H_2O was similar: results were comparable or slightly better than in cyclohexane.

Watercol™ 1910 – As reported elsewhere [11], the selectivity of 1910 differed from that of 1460. With 1910, eugenol (**16**) and vanillin (**24**) were not detected, limonene (**5**) coeluted with both solvents (cyclohexane and EtOH/H_2O), and the column failed to separate three pairs of compounds (i.e. phenylacetaldehyde (**2**)/α-ionone (**19**), estragole (**8**)/methyl octynoate (**12**), and hydroxycitronellal (**17**)/farnesol isomer b (**27b**)) (Table 2b).

Analysis of the standard mixture in cyclohexane also revealed good column performance. Most components presented tailing factors between 0.8 and 1.2; the exceptions were neral (**11**) (TF 1.6) geraniol (**13**) (TF 1.9), β-citronellol (**15**) (TF 1.9) and lyral isomer a (**24a**) (TF 1.3) and lyral isomer b (**24b**) (TF 1.5). Peak widths, expressed as σ values, ranged from 0.023 min for α-pentyl cinnamyl alcohol (**22**) and benzyl benzoate (**25**) to 0.044 min for geranial (**10**), the sole exception being lilial (**21**) whose σ was 0.093 min.

The same standard mixture solubilized in EtOH/H_2O and analyzed with 1910 column gave better results for most components. Most peaks were characterized by tailing factors ranging from 0.9 to 1.1, and some were perfectly symmetrical (TF 1.0). TF was equal or better for all components, baring neral (**11**), which had a higher TF (1.9) than in cyclohexane. Peak widths, expressed as σ values, ranged from 0.024 min for methyl eugenol (**18**) to 0.055 min for geranial (**10**), again with the exception of lilial (**21**) (0.111 min).

Again for 1910, percentage recovery vs. OV-1701 was measured using benzyl benzoate (**25**) as internal reference (Figure 2b). This column provided recoveries above 65% for all components with the standard mixture in cyclohexane, with the exception of lyral isomer a (**24a**) (25%), lyral isomer b (**24b**) (28%), farnesol isomer a (**27a**) (59%), and benzyl cinnamate (**29**) (53%). The standard mixture in EtOH/H_2O gave recoveries

below 65% only for neral (**11**) (62%), benzyl cinnamate (**29**) (53%), and lyral isomers a (**24a**) (29***%***) and b (**24b**) (37%).

3.1.2 Tuning the injection modality

The previous paragraph showed that both 1460 and 1910 columns performed correctly on the reference test mixture, with either cyclohexane or EtOH/H_2O as solvent. The main drawback was the rather poor repeatability with the EtOH/H_2O mixture, compared to that with cyclohexane (Table 3). The results indicate that the cause of this inconsistency is injection. Repeatable and non-discriminative split injection, in particular for quantitative analysis, requires the correct transfer of the sample, fully vaporized, into the injector body at the head of the column, where a narrow band must be generated. This step not only entails selecting the correct temperature, but also applying a suitable injector head pressure that is compatible with both the nature of the solvent and the liner volume and shape [18, 19]. When applied to aqueous samples, the injection conditions adopted with conventional solvents can lead to non-homogeneous sample transfer at the head of the column. This is because of the physico-chemical characteristics of water, which, under the same conditions used for conventional solvents, requires more heat and a longer time to vaporize, produces a large volume of steam and, importantly, slows the transfer to the head of the column because of its viscosity. For instance, 1.0 µL of water in the liquid state, injected at 200 kPa at 200°C, produces a volume of steam of 722 µL, while at 230°C the volume is 768 µL, whereas, under the same conditions, cyclohexane gives 120 µL and 128 µL, respectively [20]. The volume of a conventional liner with a 5 mm plug of silanized glass wool is about 800 µL, i.e. very close to the volume of steam at the two temperatures, while that of cyclohexane is within the optimal range for correct transfer.

These large steam volumes can induce randomized sample and analyte discrimination, and irregular transfer to the column, in particular affecting the quantitative performance of the injection step. Injection performance can be improved by acting on both the steam volume at the applied temperature and the liner characteristics. The volume can be reduced by increasing the pressure inside the injector, i.e. by operating in an over-pressurized split injection mode. The volume of water vapor phase produced at 350 kPa at 200°C in the injector body falls to 482 µL, thus providing better compatibility with the liner volume. In this study, most samples were solubilized in EtOH/H_2O, giving a final vapor nominal volume of 353 µL at 350 kPa and 200°C, and of 377 µL at 230°C calculated for each solvent with the dedicated Agilent calculator and mediated for the solvent mixture [20]. The liner was also adapted to the volume of steam, which was in any case above that of conventional solvents (e.g. cyclohexane), by including a double layer of silanized glass wool plug (i.e. ca 1 cm). Injection pressure of 350 kPa was chosen as it gave the best results in a series of experiments carried out with pressure ranging from 250 to 450 kPa. This pressure, applied to the 1460 and 1910 columns, with hydrogen as carrier gas, led to a very marked improvement of repeatability of the components of the allergen test mixture in EtOH/H_2O, making correct quantitative analysis possible (Table 3). The standard mixture in

cyclohexane, injected in 1460 under conventional injection conditions (200°C/200 kPa), presented RSD% calculated on six replicates ranging from 0.2% for benzyl salicylate (**28**) and benzyl cinnamate (**29**) to 6.8% for hydroxycitronellal (**17**). The same mixture in these conditions in EtOH/H_2O ranged from 11.9% for estragole (**8**) to 52.8% for lyral isomer a (**24a**) whereas when injected at high pressure (230°C/350 kPa), it gave RSD% ranging from 3.6% for α-ionone (**19**) and amyl cinnamaldehyde (**20**) to 6.2% for α-isomethyl ionone (**23**). Similar results were obtained with 1910 with the same standard mixture at 230°C/200 kPa; in cyclohexane, RSD% ranged from 1.0% for cinnamaldehyde (**3**) to 10.8% for benzyl cinnamate (**29**). In EtOH/H_2O, it ranged from 6.3% for cinnamaldehyde (**3**) to 38.0% for lyral isomer b (**24b**). The RSD% of the same sample in EtOH/H_2O, but at 200°C/350 kPa, ranged from 0.2% for linalool (**14**) and β-citronellol (**15**) to 5.5 % for lyral isomer b (**24b**).

The method was then applied to GC-MS, where helium must be used as carrier gas. To achieve the same separation, a significantly higher initial pressure (320 kPa) had to be applied to GC-MS to maintain the same performance as GC-FID, owing to helium's physico-chemical characteristics. This requirement would have implied over-pressurization of the injection chamber to at least 450 kPa, which would affect peak shape and repeatability.

The high efficiency and selectivity of both the Watercol™ columns investigated assure good results, even with the usual 10 m NB columns, which required the applied helium pressures to be reduced to values comparable to those previously optimized, i.e. 240 kPa at the head of the column, with over-pressurization at 350 kPa for one minute during injection. The results indicated a maximum RSD% of 9.4% for benzyl salicylate (**28**) with 1460, and of 6.6% for neral (**11**) with 1910. The difference of repeatability between GC-MS and GC-FID, although always acceptable, are probably justified by the different nature of the gas carrier and overpressure/pressure ratio. For both columns, the EtOH/H_2O results were often better than those with cyclohexane as main solvent.

A further consideration concerns the application of high split ratios (usually 1:100), again in view of reducing the amount of water reaching the MS detector. This can of course affect method sensitivity; however, even at high split ratios, with the applications here discussed LOD and LOQ were, in all cases, compatible with the no-declaration limits set by the EU (see tables 1 and 4).

Under these conditions, more than 700 GC runs were carried out with each of the investigated columns, with highly repeatable results, in line with the requirements of quantitative analysis (see next paragraph). The liner had to be replaced more frequently (every 200 injections) because the steam made the silanized glass wool and inner glass walls reactive towards the investigated analytes.

3.1.3 Mass spectrometry and water injection

The performance of MS detection was maintained by adopting narrow-bore columns, to limit the amount of water transferred, as well as by operating in *no-ionization* mode, during the time range of water elution as determined by GC-MS (see 2.2.5.).

3.2 Direct quantitation of suspected allergens and markers in commercial perfumes

The performance of the NB 1460 and 1910 columns on real-world samples was tested on two commercially-available perfumes. The first step entailed selecting a column that would separate and identify as many suspected allergens as possible in the test perfumes in a single run. Figure 3 reports the GC-MS patterns of the two perfumes analyzed with the two columns under study; GC-MS analyses showed that 1910 provided the most effective separation of the allergens in the investigated perfumes (Table 1 and 4), although 1460 was complementary, enabling eugenol, not detected with 1910, to be quantified, as necessary to confirm the quantitative results (table 4).

The following suspected allergens were identified: *perfume 1*: benzyl alcohol (**1**), cinnamyl alcohol (**4**), coumarin (**9**); geraniol (**13**), linalool (**14**), β-citronellol (**15**), eugenol (**16**), hydroxycitronellal (**17**), lilial (**21**), α-isomethyl ionone (**23**), lyral isomer a (**24a**), lyral isomer b (**24b**), hexyl-cinnamaldehyde (**26**), linalyl acetate (**30**); *perfume 2*: geraniol (**13**), linalool (**14**), β-citronellol (**15**), and linalyl acetate (**30**). Although not on the original list of allergens, linalyl acetate (**30**) was also studied, since it was included in the 2011 recommendation of the Scientific Committee on Consumer Safety, containing 54 compounds and isomers and some extracts [4].

Table 1 reports the figures of merit of the quantitative method of the investigated allergens for both Watercol™ columns; they are representative of the method's analytical performance. All compounds were quantified by external calibration with pure standards, using carvacrol as internal standard. The linearity of the method for all analytes was investigated over a wide range of concentrations (5-1000 mg/L) chosen in consideration of the widely differing compositions of commercial perfumes, as is indeed shown by the two investigated. In spite of the very wide concentration range considered, linearity, measured in terms of regression coefficients (R^2), was very good for all analytes with both columns, the minimum R^2 in all cases being above 0.9917 (linalyl acetate (**30**)) for 1460, and 0.9969 (lilial (**21**) and linalyl acetate (**30**)) for 1910. Similar consideration can be made for area repeatability: the maximum RSD% values (n=6) were 7.5% for lyral isomer b (**24b**) for 1460, and 7.3% for lyral isomer a (**24a**) with 1910; likewise, maximum intermediate precision was 12.4% for geraniol (**13**) for 1460, and 11.9% for lyral isomer a (**24a**) with 1910. The high values for lyral isomers may partly be due to their tailed peak shape, which might interfere with correct area determination. With both Watercol™ columns, LODs and LOQs of all components of the perfumes, diluted 1:10, were in conformity with the leave-on allergen declaration (0.01%). LODs ranged between 1.1 mg/L for coumarin (**7**) and 2.8 mg/L for geraniol (**13**) for 1460, and 1.1 mg/L for benzyl alcohol (**1**) and 2.5 mg/L for hydroxycitronellal (**17**) for 1910. LOQs were between 3.1 mg/L for hexyl cinnamaldehyde (**26**) and 7.3 mg/L

for lyral isomer a (**24a**) with 1460, and 3.2 mg/L for coumarin (**7**) and 7.2 mg/L again for lyral isomer a (**24a**) with 1910. Table 4 reports quantitative data and repeatability of the allergens identified in the two commercial perfumes investigated, by direct injection in GC-MS with both Watercol™ columns; *perfume 1* was diluted 1:10 and *perfume 2* 1:20, so as to fall within the range of linearity (5-1000mg/L) of linalyl acetate (**30**). The results with the two columns were highly consistent, the percent result differences for all analytes being in all cases below 9%, with the exception of lyral isomer a (**24a**) (11.2%). These results were confirmed by their full agreement with those obtained on the same perfumes analyzed with the reference IFRA method (data not reported).

4. Conclusions

The results show that both 1460 and 1910 narrow bore Watercol™ columns can reliably be used for the GC-MS analysis of samples in which water is the main solvent, with methods easy to apply with relatively-recent instrumentation. These columns were here successfully used for the determination of EU-regulated volatile allergens in commercial perfumes, and showed complementary selectivity; this can be very useful in cases of co-elution(s) of target analytes, which are quite frequent when analyzing complex samples. The results also indicate that the chromatographic performance of both 1910 and 1460 columns is in line with those of narrow-bore columns coated with conventional SPs, and is not affected by the direct injection of water samples. This consistency of column performance was found to be stable throughout the study, amounting to some 700 injections of water samples for each column; performance remained unvaried without affecting MS results. This reliability derives from the careful tuning of the analysis conditions, aiming to minimize the amount of water reaching columns and MS detector; this was achieved by adopting high split ratios, narrow-bore columns, and dedicated MS conditions. These developments resulted in figures of merit meeting the required sensitivity, accuracy and repeatability, and comparable to those of the methods proposed by international organization(s) [1, 2].

Watercol™ columns applied to aqueous samples comply with all specifications characteristic of routine quality control analysis of perfumes, while reducing total analysis time by a factor of about 2; they also eliminate intermediate sample preparation steps, which may be a source of discrimination between analytes, while increasing analysis time and cost. Conversely, the main limitation of direct injection of aqueous samples is the reduction of method sensitivity compared to conventional methods, involving sample preparation with high concentration capacity techniques (*e.g.* SPME), because of the lack of a concentration step of target analyte(s). The relatively high LOQs can be a limit for quantitation of minor components in leave-on products, although the direct injection procedure can be used as analytical decision maker [21] in these cases, for fast screening to decide which sample(s) need to be analyzed with methods providing better sensitivities.

Acknowledgements

The authors are indebted to MilliporeSigma (Bellefonte, PA, USA) for providing the ionic liquid columns and to Robertet SA (Grasse, France) for financial support to the laboratory. M. Minteguiaga thanks Organizzazione Internazionale Italo-Latino Americana (IILA) for providing a research fellowship in Italy

Compliance with ethical standards

Len Sidisky is an employee of MilliporeSigma (Bellefonte, PA, USA)

References

[1] DIRECTIVE 2003/15/EC of the European Parliament and of the Council of 27 February 2003.

[2] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.

[3] Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers. Fragrance Allergy in Consumers. 1999. Available from http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out98_en.pdf Assessed: June, 2019.

[4] Scientific Committee on Consumer Safety. Opinion on Fragrance allergens in cosmetic products. 2011. Available from: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf. Accessed: June, 2019.

[5] D.A. Jayawardhana, R.M. Woods, Y. Zhang, C.L. Wang, D.W. Armstrong, Rapid, Efficient Quantification of Water in Solvents and Solvents in Water Using an Ionic Liquid-Based GC Column, LCGC North Am., 30 (2012) 142-158.

[6] E. R. Kuhn, Water injections in GC − how wet can you get?, LCGC North Am. 20 (2002) 475-478

[7] L.A. Frink, D.W. Armstrong, The utilisation of two detectors for the determination of water in honey using headspace gas chromatography, Food Chem., 205 (2016) 23-27.

[8] L.A. Frink, D.W. Armstrong, Water Determination in Solid Pharmaceutical Products Utilizing Ionic Liquids and Headspace Gas Chromatography, J. Pharm. Sci-Us., 105 (2016) 2288-2292.

[9] L.A. Frink, C.A. Weatherly, D.W. Armstrong, Water determination in active pharmaceutical ingredients using ionic liquid headspace gas chromatography and two different detection protocols, J. Pharmaceut. Biomed., 94 (2014) 111-117.

[10] https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/General_Information/1/T415156 -watercol -capillary-gc-columns.pdf. Accessed: June, 2019.

[11] 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., 410 (2018) 4657-4668.

[12] B. Sgorbini, C. Cagliero, S. Acquadro, A. Marengo, C. Cordero, E. Liberto, C. Bicchi, P. Rubiolo, Evaluation of volatile bioactive secondary metabolites transfer from medicinal and aromatic plants to herbal teas: Comparison of different methods for the determination of transfer rate and human intake, J. Chromatogr. A, 1594 (2019) 173-180.

[13] IFRA (International Fragrance Association). GC/MS quantitation of potential fragrance allergens in fragrance compounds. 2007.

[14] IFRA (International Fragrance Association) - Analytical Working Group - Analytical method to quantify 57 suspected allergens (and isomers) in ready to inject fragrance materials by Gas Chromatography and Mass Spectrometry - November 15th, 2016.

[15] E. Belhassen, D. Bressanello, P. Merle, E. Raynaud, C. Bicchi, A. Chaintreau, C. Cordero, Routine quantification of 54 allergens in fragrances using comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry with dual parallel secondary columns. Part I: Method development, Flavour Frag. J., 33 (2018) 63-74.

[16] K. Grob, G. Grob, K.J. Grob, Testing capillary gas chromatographic columns, J. Chromatogr., 219 (1981) 13-20.

[17] L.M. Blumberg, M.S. Klee, Metrics of separation in chromatography, J. Chromatogr. A 933 (2001) 1-11

[18] K. Grob, Split and Splitless Injection for Quantitative Gas Chromatography, Wiley VCH Weinheim (Germany), 2001.

[19] M. Biedermann, Inlet system and sample introduction, in: K. Dettmer-Wilde, W. Engewald (Eds.) Practical Gas Chromatography - A Comprehensive Reference, Springer-Verlag Berlin Heidelberg, 2014, pp. 161-204.

[20] https://www.agilent.com/en/support/gas-chromatography/gccalculators. Accessed: June, 2019.

[21] P. Sandra, F. David, B. Tienpont, From CGC-MS to MS-based analytical decision makers, Chromatographia, 60 (2004) S299-S302.

Captions to figures:

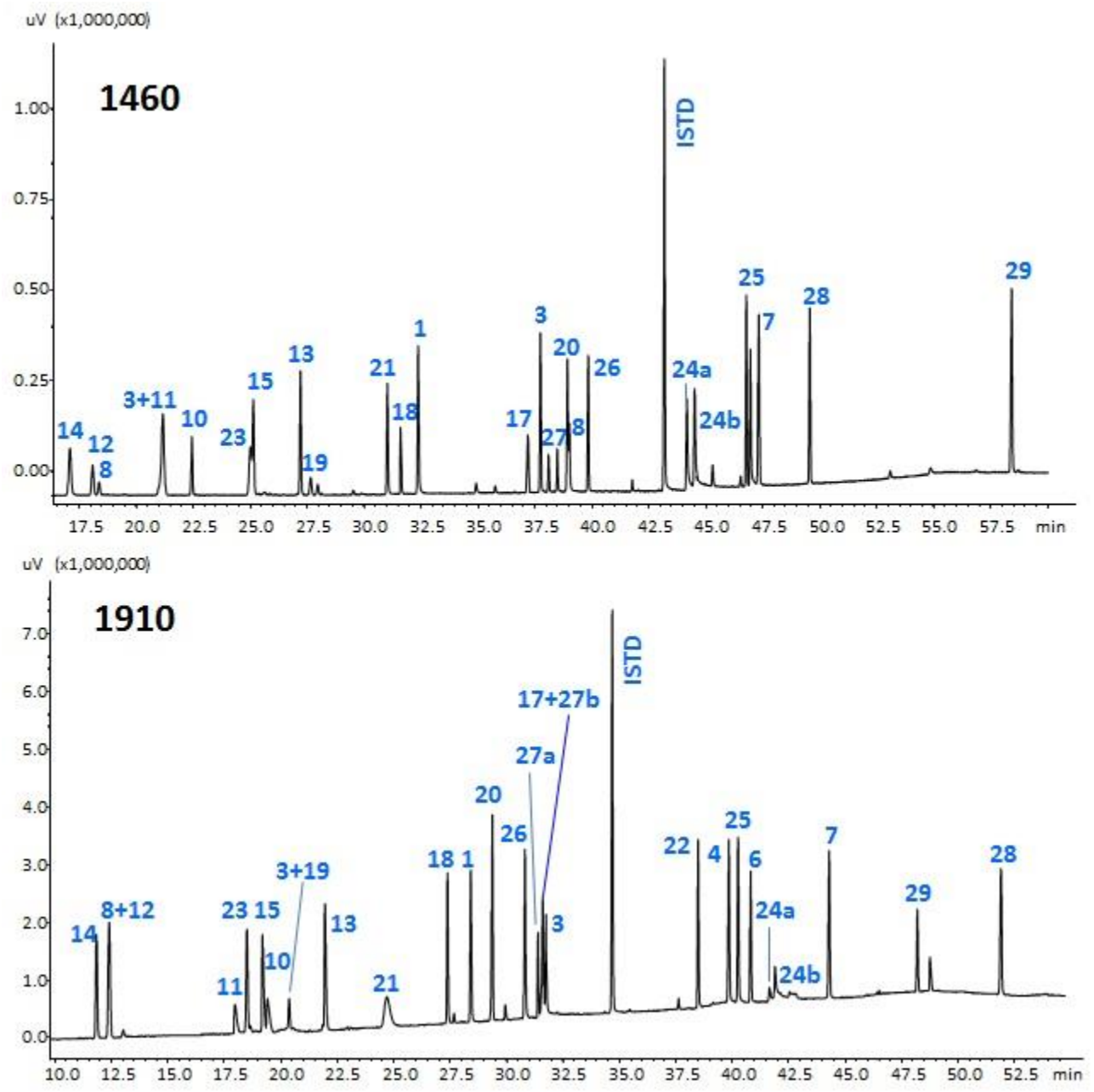
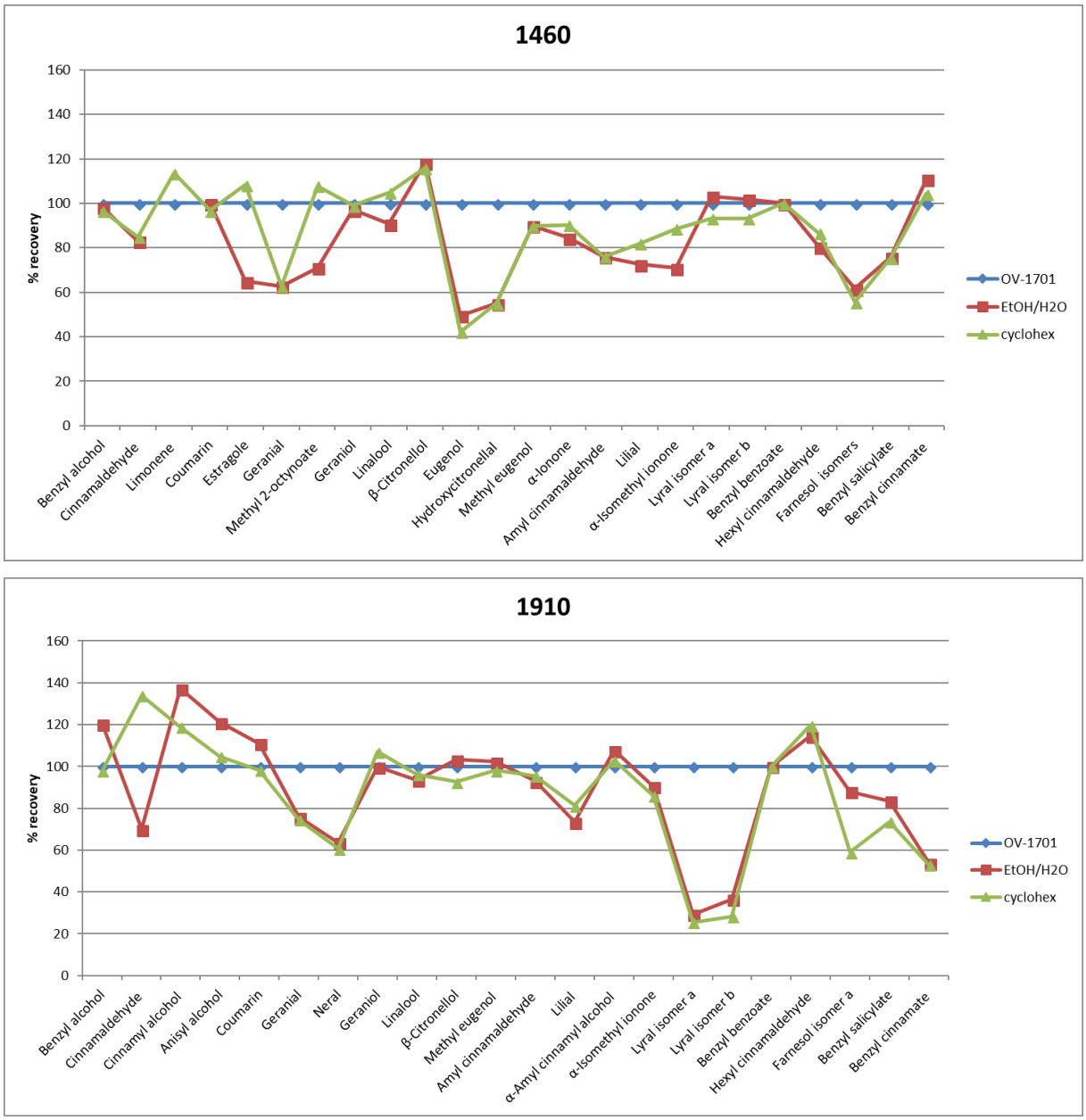


Figure 1 - GC-FID patterns of the allergen standard mixture diluted in 1:1 EtOH/H_2O analyzed with Watercol™ 1460, and 1910. For analysis conditions see text. Peak identification: **1.** benzyl alcohol, **2.** phenylacetaldehyde, **3.** cinnamaldehyde, **4.** cinnamyl alcohol, **5.** limonene, **6.** anisyl alcohol, **7.** coumarin, **8.** estragole, **9.** vanillin, **10.** geranial, **11.** neral, **12.** methyl-2-octynoate, **13.** geraniol, **14.** linalool, **15.** β-citronellol, **16.** eugenol, **17.** hydroxycitronellal; **18.** methyl eugenol, **19.** α-ionone, **20.** amyl cinnamaldehyde, **21.** lilial, **22.** α-pentyl cinnamyl alcohol, **23.** α-isomethyl ionone, **24a.** lyral isomer a, **24b.** lyral isomer b, **25.** benzyl benzoate, **26.** hexyl cinnamaldehyde, **27.** farnesol isomers, **28.** benzyl salycilate, and **29.** benzyl cinnamate.

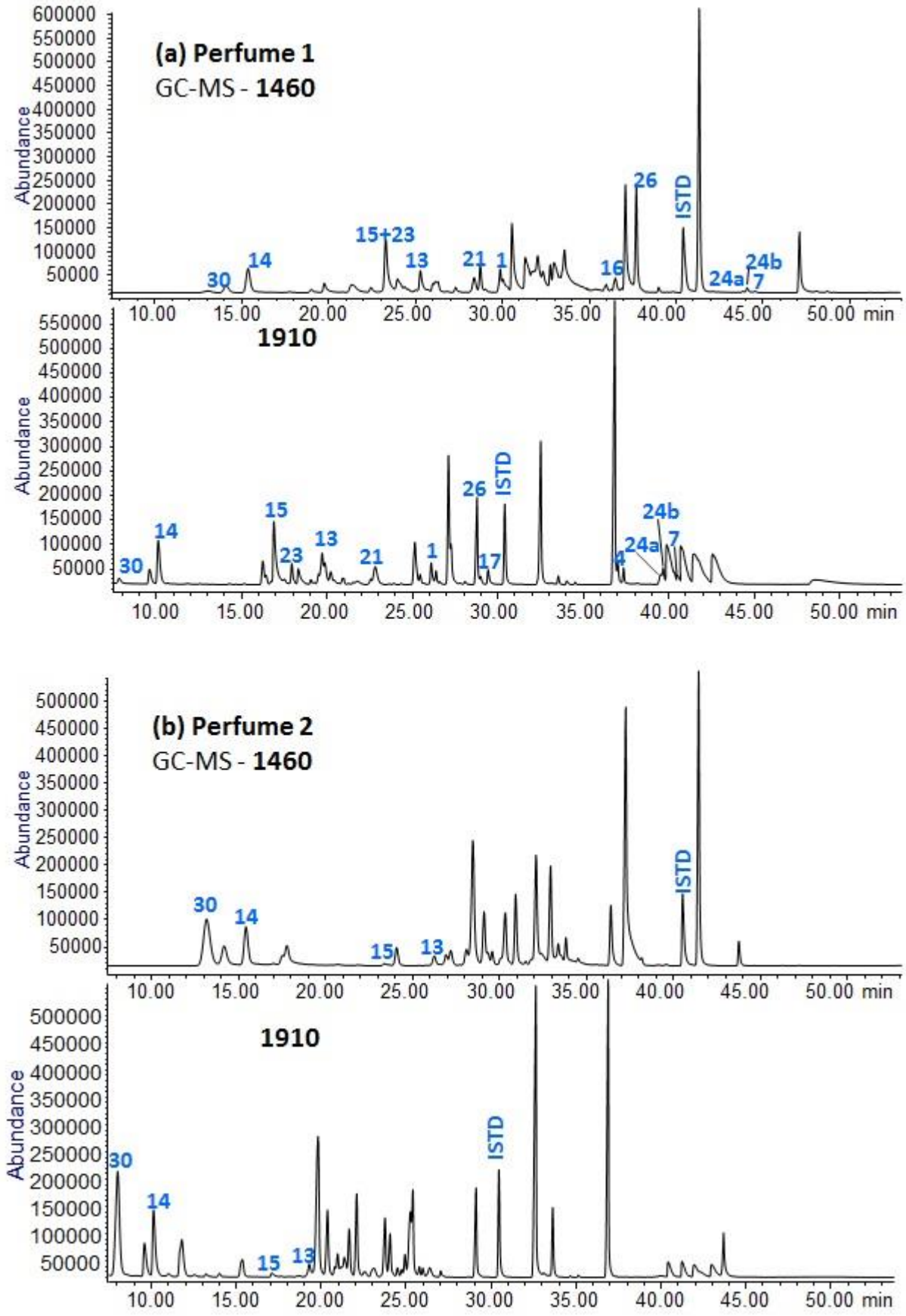


499

500

501 **Figure 2** - Recovery of suspected allergens, calculated from the normalized absolute area of each analyte,

502 with Watercol™ 1460 and 1910, *versus* OV-1701 columns, taken as reference.



503

504 **Figure 3** - GC-MS patterns of perfume 1 (a) and perfume 2 (b) on Watercol™ 1460 and 1910. For analysis

505 conditions see text. Peak identification: **1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.**

linalool, **15.** β-citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** lilial, **23.** α-isomethyl ionone, **24a.** and **24b.** lyral isomers, **26.** hexyl cinnamaldehyde and **30**. linalyl acetate

Captions to tables

Table 1 - Figures of merit of the method applied to quantitation of the allergens in perfumes 1 and 2. Lin.:

			1460					1910				
N	**Compound**	**Selected ions**	**Lin. R^2**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**	**Rep. %RSD**	**I.P. %RSD**	**Lin. R^2**	**LOD mg L^{-1}**	**LOQ mg L^{-1}**	**Rep. %RSD**	**I.P. %RSD**
1	Benzyl alcohol	79(Q) 65/91/108	0.9931	1.8	6.9	4.7	7.8	0.9982	1.1	3.4	2.2	6.6
4	Cinnamyl alcohol	92(Q) 105/115/134	*ND*	*ND*	*ND*	*ND*	*ND*	0.9976	1.3	4.1	7.1	11.6
7	Coumarin	118(Q) 63/89/146	0.9992	1.1	3.8	1.3	4.3	0.9994	1.4	3.2	1.0	8.3
13	Geraniol	69(Q) 93/123/136	0.9935	2.8	7.1	7.4	12.4	0.9973	2.1	6.6	5.7	9.8
14	Linalool	71(Q) 93/121/136	0.9959	1.7	6.4	2.3	4.6	0.9979	2.1	6.6	5.7	9.2
15	β-Citronellol	69(Q) 81/95/109	0.9980	2.1	6.6	2.0	5.5	0.9980	1.7	5.7	2.8	7.4
16	Eugenol	164(Q) 91/131/149	0.9973	1.3	4.7	2.5	6.3	*ND*	*ND*	*ND*	*ND*	*ND*
17	Hydroxycitronellal	59(Q) 71/81/95	0.9975	2.6	6.6	4.3	8.7	0.9974	2.5	7.3	4.3	10.2
21	Lilial	189(Q) 131/147/204	0.9944	2.3	7.1	2.6	4.8	0.9969	1.8	6.3	2.4	7.4
23	α-i-Methyl ionone	135(Q) 123/150/191	0.9978	2.1	6.6	1.2	5.3	0.9979	1.3	4.2	1.6	5.9
24a	Lyral isomer a	105(Q) 118/136/163	0.9932	2.6	7.3	6.7	9.6	0.9971	2.5	7.2	7.3	11.9
24b	Lyral isomer b	136(Q) 93/149/192	0.9938	2.4	7.1	7.5	10.2	0.9977	2.3	6.7	5.1	9.7
26	C_6 Cinnamaldehyde	129(Q) 117/145/216	0.9980	1.4	3.1	1.4	4.8	0.9988	1.7	3.4	3.7	7.6
30	Linalyl acetate	93(Q) 80/121/136	0.9917	2.1	6.8	1.0	6.5	0.9969	2.3	6.6	6.8	9.8

linearity, R^2: regression coefficient; LOD: limit of detection; LOQ: limit of quantitation; ND: not detected; Rep.: repeatability, calculated at 50 mg L^{-1} (n= 6); I.P.: Intermediate Precision calculated at 50 mg L^{-1}

Table2 - Figures of merit (retention time, tailing factor and σ of the peak width) of the two Watercol™ columns investigated, calculated from the analysis of the allergen standard mixture in cyclohexane and EtOH/H_2O with; *ND*: not detected, *NM*: not measurable (partial coelutions); a) 1460 NB column 15 m, detector FID, carrier: H_2; coelutions: *coel 1*: phenylacetaldehyde (**2**)/neral (**11**); b) 1910 NB column 15 m, detector FID, carrier: H_2; coelutions: *coel 2*: phenylacetaldehyde (**2**)/α-ionone (**19**); *coel 3*: estragole (**8**)/methyl octynoate (**12**); *coel 4*: hydroxycitronellal (**17**)/farnesol isomer a (**27a**)

2a	**1460**	**Cyclohexane**		**EtOH/H2O 1:1**

N	Compounds	Ret. time (min)	Tailing factor	σ (min)		Ret. Time (min)	Tailing factor	σ (min)
1	Benzyl alcohol	32.54	1.2	0.027		32.52	1.2	0.027
2	Phenylacetaldehyde	21.14	*coel 1*	*coel1*		21.12	*coel 1*	*coel 1*
3	Cinnamaldehyde	37.92	1.0	0.023		37.90	1.0	0.025
4	Cinnamyl alcohol	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
5	Limonene	3.09	1.1	0.133		*NM*	*NM*	*NM*
6	Anisyl alcohol	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
7	Coumarin	47.59	1.0	0.028		47.58	1.0	0.028
8	Estragole	18.51	1.0	0.039		18.47	1.0	0.035
9	Vanillin	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
10	Geranial	22.57	0.9	0.040		22.56	0.9	0.037
11	Neral	21.14	*coel 1*	*coel 1*		21.12	*coel 1*	*coel 1*
12	Methyl 2-octynoate	18.22	1.0	0.044		18.18	1.0	0.042
13	Geraniol	27.36	1.0	0.027		27.34	1.0	0.027
14	Linalool	17.22	1.0	0.051		17.19	1.0	0.051
15	β-Citronellol	25.29	*NM*	*NM*		25.26	*NM*	*NM*
16	Eugenol	39.21	*NM*	*NM*		39.17	*NM*	*NM*
17	Hydroxycitronellal	37.36	1.1	0.021		37.32	1.0	0.021
18	Methyl eugenol	31.74	1.1	0.026		31.76	1.1	0.026
19	α-Ionone	27.81	0.9	0.036		27.78	1.0	0.038
20	Amyl cinnamaldehyde	38.11	*NM*	*NM*		38.09	*NM*	*NM*
21	Lilial	31.18	1.0	0.025		31.16	1.1	0.026
22	α-Pentyl cinnamyl alcohol	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
23	α-Isomethyl Ionone	25.16	*NM*	*NM*		25.13	*NM*	*NM*
24a	Lyral isomer a	44.37	2.0	0.043		44.35	1.2	0.028
24b	Lyral isomer b	44.72	2.2	0.043		44.69	1.3	0.029
25	Benzyl benzoate	47.05	1.0	0.023		47.03	1.0	0.023
26	Hexyl cinnamaldehyde	40.03	1.0	0.023		40.00	1.0	0.024
27a 27b	Farnesol isomer a Farnesol isomer b	38.67	1.1	0.22		38.64	1.1	0.023
28	Benzyl salycilate	49.83	1.0	0.024		49.82	0.9	0.024
29	Benzyl cinnamate	58.67	1.0	0.030		58.67	0.9	0.031

2b	**1910**	**Cyclohexane**				**EtOH/H2O 1:1**		
N	**Compounds**	**Ret. time (min)**	**Tailing factor**	**σ (min)**		**Ret. time (min)**	**Tailing factor**	**σ (min)**
1	Benzyl alcohol	28.59	1.1	0.028		28.62	1.1	0.028
2	Phenylacetaldehyde	20.57	*coel 2*	*coel 2*		20.56	*coel 2*	*coel 2*
3	Cinnamaldehyde	31.82	*NM*	0.040		31.96	*NM*	0.040
4	Cinnamyl alcohol	40.05	1.1	0.026		40.07	1.0	0.027

5	Limonene	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
6	Anisyl alcohol	41.02	1.1	0.026		41.04	1.1	0.026
7	Coumarin	44.52	1.0	0.028		44.52	1.0	0.029
8	Estragole	12.88	*coel 3*	*coel 3*		12.58	*coel 3*	*coel 3*
9	Vanillin	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
10	Geranial	19.43	*NM*	0.044		19.60	*NM*	0.055
11	Neral	18.03	1.6	0.042		18.15	1.9	0.050
12	Methyl 2-octynoate	12.88	*coel 3*	*coel 3*		12.58	*coel 3*	*coel 3*
13	Geraniol	22.05	1.9	0.038		22.15	1.5	0.032
14	Linalool	11.98	1.2	0.038		12.01	1.1	0.035
15	β-Citronellol	19.23	1.9	0.043		19.37	1.9	0.040
16	Eugenol	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*
17	Hydroxycitronellal	31.66	*coel 4*	*coel 4*		31.82	*coel 4*	*coel 4*
18	Methyl eugenol	27.59	1.1	0.026		27.59	1.0	0.024
19	α-Ionone	20.58	*coel 2*	*coel 2*		20.56	*coel 2*	*coel 2*
20	Amyl cinnamaldehyde	29.58	1.0	0.027		29.58	0.9	0.025
21	Lilial	24.93	1.2	0.093		24.90	1.2	0.111
22	α-Pentyl cinnamyl alcohol	38.68	1.1	0.023		38.71	1.1	0.024
23	α-Isomethyl Ionone	18.70	1.2	0.035		18.69	1.1	0.034
24a	Lyral isomer a	41.86	1.3	0.029		41.88	1.2	0.029
24b	Lyral isomer b	42.11	1.5	0.032		42.13	1.5	0.034
25	Benzyl benzoate	40.48	1.0	0.023		40.48	1.1	0.025
26	Hexyl cinnamaldehyde	31.01	0.8	0.030		31.02	1.1	0.027
27a	Farnesol isomer a	31.49	1.2	0.036		31.60	1.2	0.027
27b	Farnesol isomer b	31.66	*coel 4*	*coel 4*		31.82	*coel 4*	*coel 4*
28	Benzyl salycilate	52.14	0.9	0.031		52.14	1.0	0.032
29	Benzyl cinnamate	48.45	1.0	0.026		48.45	1.0	0.026

Table 3 - GC-FID and GC-MS injection repeatability (RSD%) of the Watercol™ columns, calculated through analysis of the allergen standard mixture (n=6). Solvents: cyC_6: cyclohexane, Et-H_2O: ethanol/water 1:1; CI: conventional injection, OpIn: overpressurized injection (H_2: 350 kPa, He: 350 kPa); *ND*: not detected, *NM*: not measurable (co-elutions)

RSD%		**GC-FID** col. length: **15 m**; carrier gas: **H_2**							**GC-MS** col. length: **10 m**; carrier gas: **He**			
		1460			**1910**				**1460**		**1910**	
	Compounds	**cyC_6 CI**	**EtOH/H_2O CI**	**EtOH/H_2O OpIn**	**cyC_6 CI**	**EtOH/H_2O CI**	**EtOH/H_2O OpIn**		**cyC_6 CI**	**EtOH/H_2O OpIn**	**cyC_6 CI**	**EtOH/H_2O OpIn**
1	Benzyl alcohol	4.3	42.2	5.0	3.6	11.2	0.8		13.6	7.1	6.8	1.9
2	Phenylacetaldehyde	*NM*	*NM*	*NM*	*NM*	*NM*	*NM*		*NM*	*NM*	*NM*	*NM*

3	Cinnamaldehyde	0.5	23.9	4.1	1.0	6.3	0.8		3.1	0.7	8.9	2.1
4	Cinnamyl alcohol	*ND*	*ND*	*ND*	2.4	22.8	0.4		*ND*	*ND*	4.6	3.4
5	Limonene	1.2	*NM*	*NM*	*ND*	*ND*	*ND*		*NM*	*NM*	*ND*	*ND*
6	Anisyl alcohol	*ND*	*ND*	*ND*	2.8	34.6	0.8		*ND*	*ND*	5.4	3.8
7	Coumarin	1.6	48.8	5.3	2.5	27.7	0.6		5.6	0.8	4.3	0.3
8	Estragole	0.3	11.9	5.0	*NM*	*NM*	*NM*		9.8	9.2	*NM*	*NM*
9	Vanillin	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*		*ND*	*ND*	*ND*	*ND*
10	Geranial	5.4	18.8	4.7	6.5	18.2	1.3		4.3	7.6	10.0	6.5
11	Neral	*NM*	*NM*	*NM*	2.6	34.0	0.9		*NM*	*NM*	3.3	6.6
12	Methyl 2-octynoate	0.6	20.0	4.6	*NM*	*NM*	*NM*		10.7	8.6	*NM*	*NM*
13	Geraniol	0.4	28.4	4.2	3.3	10.7	0.7		2.8	4.7	4.1	5.3
14	Linalool	2.6	17.9	5.0	4.6	24.8	0.2		3.6	4.3	5.4	5.6
15	β-Citronellol	0.9	22.6	3.8	3.9	14.9	0.2		3.6	7.8	7.7	4.4
16	Eugenol	0.2	36.6	4.2	ND	ND	ND		*NM*	*NM*	*ND*	*ND*
17	Hydroxycitronellal	6.8	24.5	4.2	*NM*	*NM*	*NM*		5.1	10.5	*NM*	*NM*
18	Methyl eugenol	4.3	32.8	4.9	2.7	9.4	0.7		2.9	2.0	6.0	3.3
19	α-Ionone	4.0	19.9	3.6	*NM*	*NM*	*NM*		2.7	5.6	*NM*	*NM*
20	C_5 cinnamaldehyde	5.5	30.0	3.6	1.9	11.2	0.8		2.6	3.7	4.8	4.0
21	Lilial	0.7	27.5	4.0	2.8	10.5	2.2		1.1	5.0	3.8	1.1
22	α-C_5 cinnamyl alcohol	*ND*	*ND*	*ND*	1.8	11.8	1.2		*ND*	*ND*	2.2	5.5
23	α-Isomethyl Ionone	4.6	16.4	6.2	2.5	17.1	0.6		3.2	6.4	2.5	6.0
24a	Lyral isomer a	6.0	52.8	4.6	4.1	28.9	4.5		9.1	7.5	2.9	5.9
24b	Lyral isomer b	1.2	46.0	4.8	8.7	38.0	5.5		1.7	2.7	3.9	3.9
25	Benzyl benzoate	0.9	34.9	3.8	1.1	8.3	1.3		6.2	5.4	2.1	3.5
26	C_6-cinnamaldehyde	0.3	24.8	4.5	1.2	10.3	1.2		1.7	1.9	4.1	3.5
27a	Farnesol isomer a	0.5	30.9	4.3	1.9	12.4	2.3		3.9	9.1	13.2	3.6
27b	Farnesol isomer b				*NM*	*NM*	*NM*				*NM*	*NM*
28	Benzyl salicylate	0.2	36.8	3.9	5.3	12.2	2.8		6.5	9.4	5.8	4.7
29	Benzyl cinnamate	0.2	42.5	4.5	10.8	11.4	3.4		0.6	3.5	5.0	5.9

Table 4 - Quantitative results and repeatability of analysis of allergens in the two investigated commercial perfumes, by direct injection in GC-MS with the two Watercol™ columns. Abs. am.: absolute amount; repeatability (SD, RSD%): n = 6, Perfume 1*: dilution 1:10; Perfume 2**: dilution 1:20; *NM*: not measurable; *ND*: not detectable. Δ %: ($Q_{1910} - Q_{1460}/Q_{1910}$)%

			1910				1460			1910/1460
		Analyte	Abs. am. mg/L (ppm)	SD	RSD%		Abs. am. mg/L (ppm)	SD	RSD%	Δ %
Per fu	1	Benzyl alcohol	*2195*	47	2.1		*2280*	114	5.0	-3.7

	4	Cinnamyl alcohol	*1169*	14	1.2		*ND*	*ND*	*ND*	*NM*
	7	Coumarin	*182*	3	1.8		*168*	9	6.2	7.7
	13	Geraniol	*3870*	62	1.6		*3628*	145	4.0	6.7
	14	Linalool	*4820*	84	1.7		*4883*	231	4.7	-1.3
	15	β-Citronellol	*8110*	145	1.8		*7507*	553	7.4	8.0
	16	Eugenol	*ND*	*ND*	*ND*		*1349*	93	6.9	*NM*
	17	Hydroxycitronellal	*1998*	17	0.9		*ND*	*ND*	*ND*	*NM*
	21	Lilial	*1966*	14	0.7		*1860*	69	3.7	5.7
	23	α-*i*-Methyl ionone	*485*	4	0.9		*527*	48	9.0	7.9
	24a	Lyral isomer a	*1624*	32	2.0		*1492*	102	7.3	8.9
	24b	Lyral isomer b	*1267*	15	1.1		*1146*	62	5.4	10.5
	26	C_6-cinnamaldehyde	*5172*	67	1.3		*5114*	292	5.7	1.1
	30	Linalyl acetate	*860*	50	5.8		*896*	63	7.1	-4.0
Perfume 2**	13	Geraniol	*1743*	7	0.4		*ND*	*ND*	*ND*	*NM*
	14	Linalool	*6748*	129	1.9		*6174*	286	4.6	9.3
	15	β-Citronellol	*649*	13	2.0		*689*	49	7.2	-5.8
	30	Linalyl acetate	*14558*	247	1.7		*15717*	683	4.3	-7.4

537