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(Article begins on next page)

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

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27 **Abstract**

28

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

41

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

44

45

46 **1. Introduction**

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

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

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

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

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

104

105 **2. Material and Methods**

106 2.1 Samples and chemicals

107 Column performance was evaluated with the Grob Test [16] (Merck, Milan, Italy). Stock solutions of the 24
108 volatile allergens and 5 other related compounds, at 5000 mg/L in cyclohexane, and in a 1:1 ethanol/water
109 mixture (hereafter EtOH/H₂O), were prepared from pure standards (all from Merck, Milan, Italy). Deionized
110 water was obtained from a Milli-Q purification system, Millipore, Merck, Milan, Italy). The mixtures of volatile
111 allergens and related compounds included: **1.** benzyl alcohol (CAS: 100-51-6), **2.** phenylacetaldehyde (CAS:
112 122-78-1), **3.** cinnamaldehyde (CAS: 104-55-2), **4.** cinnamyl alcohol (CAS: 104-54-1), **5.** limonene (CAS: 138-
113 86-3), **6.** anisyl alcohol (CAS: 105-13-5), **7.** coumarin (CAS: 91-64-5), **8.** estragole (CAS: 140-67-0), **9.** vanillin
114 (CAS: 121-33-5), **10.** geranial (CAS: 5392-40-5), **11.** neral (CAS: 106-26-3), **12.** methyl-2-octynoate (CAS: 111-
115 12-6), **13.** geraniol (CAS: 106-24-1), **14.** linalool (CAS: 78-70-6), **15.** β-citronellol (CAS: 106-22-9), **16.** eugenol

116 (CAS: 97-53-0), **17.** hydroxycitronellal (CAS: 107-75-5); **18.** methyl eugenol (CAS: 93-15-2), **19.** α -ionone (CAS:
117 127-41-3), **20.** amyl cinnamaldehyde (CAS: 122-40-7), **21.** lilial (CAS: 80-54-6), **22.** α -pentyl cinnamyl alcohol
118 (CAS 101-85-9), **23.** α -isomethyl ionone (CAS: 127-51-5), **24a.** lylal isomer a (CAS: 51414-25-6) **24b.** lylal
119 isomer b (CAS: 31906-04-4), **25.** benzyl benzoate (CAS: 120-51-4), **26.** hexyl cinnamaldehyde (CAS: 101-86-0),
120 **27.** farnesol isomers (CAS: 106-28-5), **28.** benzyl salicylate (CAS: 118-58-1) and **29.** benzyl cinnamate (CAS:
121 103-41-3). The working solutions (30 mg/L for each analyte) were prepared by suitable dilution of the stock
122 solution in the same solvents and directly injected into the GC systems.

123 Two commercial perfumes labelled as “eau de toilette” (perfume 1 and perfume 2) were obtained from a
124 local supermarket and injected into the GC and GC-MS systems after dilution at 1:10 or 1:20 in EtOH/H₂O
125 solution. Standard mixtures of the following compounds were prepared for the quantitation of allergens in
126 these perfumes: **1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.** linalool, **15.** β -
127 citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** lilial, **23.** α -isomethyl ionone, **24a.** and **24b.** lylal isomers,
128 **26.** hexyl cinnamaldehyde and **30.** linalyl acetate (CAS: 115-95-7). Linalyl acetate was also included because
129 it is comprised in the extended EU list of 54 compounds to be monitored [14]. Carvacrol (CAS: 499-75-29)
130 was provided by Merck and was used as internal standard (ISTD) for quantitation because it does not co-
131 elute with any other perfume component under the analysis conditions applied.

132

133 2.2 Analysis conditions

134 2.2.1 Instrumental set-up

135 A Shimadzu 2010 GC-FID-TCD system equipped with an autosampler AOC-20i and combined with GC Solution
136 Version 2.30.00 SU6 software (Shimadzu Co., Kyoto, Japan) was adopted to optimize injection conditions for
137 aqueous samples. The two detectors were used separately. GC-MS analyses were carried out on an Agilent
138 6890N GC coupled to a 5975 MSD provided with a 7683B autosampler and a ChemStation Version
139 E.02.02.1431 data processing system (Agilent Technologies, Santa Clara, CA).

140

141 2.2.2 Columns

142 Four non-bonded narrow-bore (NB) capillary Watercol™ columns from MilliporeSigma (Bellefonte, PA, USA)
143 were used: two 1460 [Tri-(tripropylphosphoniumhexanamido)-triethylamine-trifluoromethane sulfonate;
144 T_{max}: 260°C] and two 1910 [1,11-Di-(3-hydroxyethylimidazolium)-3,6,9-trioxaundecane trifluoro methane
145 sulfonate; T_{max}: 180°C]. Column characteristics: d_c : 0.10 mm; d_f : 0.08 μ m; length: 10 and 15 m. The
146 investigated NB columns were specifically prepared for this study, and submitted to a dedicated property
147 deactivation procedure. The two types of column are characterized by different operative temperatures:
148 1460 operates from 60°C to 260°C, and 1910 between 40°C and 180°C. A NB OV-1701 column (length: 10 m,
149 d_c : 0.10 mm; d_f : 0.10 μ m) was used as reference.

150

151 2.2.3. Optimization of injection conditions for aqueous samples

152 Injection conditions were optimized on GC-FID with 15 m capillary columns, on the standard mixture of the
153 29 allergens and related compounds (hereafter allergens) with hydrogen as carrier gas. The investigated
154 conditions were: i) liners without or with 0.5 or 1cm glass wool plug, and internal volume of about 800 μ L for
155 GC-FID-TCD (Agilent Crosslab P/N: 8001-0104); ii) injection pressure: 200 kPa or overpressurized from 250 to
156 450 kPa; and iii) split ratios: 50, 70 and 100. The optimized conditions were then adapted to GC-MS equipped
157 with 10 m columns and helium as carrier gas, and with a liner with internal volume about 900 μ L (Agilent
158 P/N: 5190-2293). GC analyses were carried out with syringes for injection provided with 5.1 mm needles and
159 liners filled with 1 cm glass wool plug, thus assuring for both GC-FID and GC-MS systems, a distance of at
160 least 3 cm between the needle tip and the glass wool plug.

161

162 2.2.4. GC-FID-TCD conditions

163 GC-FID-TCD analyses (with 15 m columns) were carried out under the following conditions: oven temperature
164 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.
165 Injector temperature: 230°C for 1460; 200°C for 1910, detector temperature (FID or TCD): 230°C. Sampling
166 rate for both detectors: 40 msec. Carrier gas: H₂ (FID), He (TCD); column flow: 0.4 mL/min in both cases; initial
167 head pressure: 199 kPa for H₂ and 332 kPa for He. Split ratio: 1:100 (if not specified otherwise). TCD current:
168 60 mA, signal polarity: positive. TCD make-up flow: 8.0 mL/min.

169

170 2.2.5. GC-MS Conditions

171 GC-MS analyses (with 10 m columns) were carried out under the following conditions: oven temperature:
172 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
173 temperature: 230°C for 1460; 200°C for 1910. MS interface temperature: 230°C in both cases. The 1910
174 column was connected to the MS through a post-column of deactivated fused silica (0.5 m x 0.10 mm d_c)
175 (Mega, Legnano, Italy) to make it compatible with the higher interface temperature. Injection mode: split;
176 ratio: 1:100; injection volume: 1.0 μ L. Carrier gas: He; column flow: 0.4 mL/min. Initial head pressure (He):
177 240 kPa. An overpressure injection (pulsed split, He) of 350 kPa for 1.0 min was applied for the aqueous
178 samples. Ion source and analyzer (quadrupole) temperatures: 230°C and 150°C, respectively. Ionization
179 mode: electron impact at 70 eV; acquisition mode: scan (35-350 m/z). Solvent delay: 3.0 min for samples in
180 cyclohexane. For aqueous samples, a solution 1/10 of the perfume 1 in EtOH/H₂O was injected once into
181 each Watercol™ column, with MS scan range set between 17 and 350 m/z, to establish the elution time
182 interval of the H₂O peak. This value was then used to set the time point at which to switch off the ion-source
183 filament (as solvent delay) in routine quantitative analyses, resulting in a solvent delay of 5.00 min for 1460,
184 and 7.40 min for 1910.

185

186 2.2.6. Analyte identification and quantitation

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

190 The volatile allergens identified in the perfumes were quantified by building up a calibration curve for each
191 of the 14 target components (**1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.** linalool,
192 **15.** β -citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** linal, **23.** α -isomethyl ionone, **24a.** lylal isomers a,
193 **24b.** lylal isomers b, **26.** hexyl cinnamaldehyde and **30.** linalyl acetate), by injecting a set of analyte standard
194 solutions, containing 5, 10, 50, 100, 200, 500 and 1000 mg/L of each compound diluted in EtOH/H₂O. Stock
195 solutions of each analyte were therefore prepared at 10.0 mg/mL in 95% EtOH and suitably diluted to the
196 required levels for calibration. A stock solution of carvacrol (ISTD) at 100 mg/mL in EtOH/H₂O was used to
197 evaluate the instrumental repeatability, adding 5.0 μ L of it to all samples to achieve a final concentration of
198 50 mg/L.

199 The analytes investigated were quantified through the area of one target ion, obtained in extract ion mode
200 (EIM) from the TIC-GC-MS data; two qualifiers, again obtained in EIM, were used for identification. Target
201 ion and qualifiers, selected from among the diagnostic fragments of each analyte, are reported in Table 1.
202 The method linearity was determined within the above concentration range, providing a determination
203 coefficient R² above 0.99. Limit of detection (LOD) and limit of quantitation (LOQ) of the method for each
204 analyte were obtained by injecting the quantitation standard mixture at 1, 5, and 10 mg/L solubilized in
205 EtOH/H₂O. LOD was calculated from the average "peak to peak" noise values sampled in the region of elution
206 of each analyte in the chromatogram, with a coverage factor of 3. LOQ was the lowest concentration for
207 which instrumental response integration reported an RSD%, across replicate analyses, below 20%. The
208 repeatability of the system was determined by 6 consecutive injections of a solution of all analytes at 50
209 mg/L, under the above conditions. Intermediate precision was determined by injecting the same solutions
210 every 3 weeks over a period of 3 months.

211

212 **3. Results and discussion**

213 Routine quantitative GC-MS analysis of target compounds in aqueous samples by direct injection entails re-
214 evaluating and tuning the three main steps of the analytical procedure, since water as solvent can i) affect
215 column performance, ii) interfere with correct injection and iii) interfere with a reliable MS response. The
216 analytical procedure has therefore been adapted to aqueous samples to obtain reliable, linear, and
217 repeatable results. Analyses were carried out in 1:1 EtOH/H₂O solution chosen as a ratio of compromise
218 due to the widely variable composition of the products of the fragrance field, that range from a highly
219 predominant percentage of ethanol in perfumes (eau de parfum, eau de toilette etc) to high percentage of
220 water in the perfumed waters.

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

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

228

229 3.1 Evaluation of column performances and optimization of GC-MS conditions

230 3.1.1 Watercol™ column performance

231 The performance stability to injection of aqueous samples, of columns coated with the two IL SPs
232 investigated, was already shown to be very high in previous work [11], by monitoring the consistency of water
233 retention indices after repeated injection (60) of EtOH/H₂O. These results were here confirmed, by analyzing
234 the above mixture consecutively for 15 times with the narrow-bore columns object of this study, by GC-TCD.
235 Water and ethanol retention times were confirmed to be very stable and repeatable (data not shown). The
236 standard mixture of 29 compounds of interest here, 24 of them included on the EU list [2], solubilized in
237 cyclohexane and in 1/1 EtOH/H₂O, was analyzed with both 1460 and 1910 columns, to evaluate column
238 performance. The consistency of analyte retention times, independently of the main solvent applied
239 (cyclohexane or EtOH/H₂O), offers a further indication of column stability (Table 2). Figure 1 reports the GC-
240 FID patterns of the allergen standard mixture, analyzed with Watercol™ 1460 and 1910. Table 2a and 2b
241 reports tailing factors and σ values of the peak width of each analyte (where the peak width at the base line
242 is 4σ) [17], after injection of standard solutions in cyclohexane and EtOH/H₂O into the two columns, while
243 Figure 2 shows recovery, measured as the ratio of the area of each analyte of the standard mixture in the
244 two solvents analyzed with the two columns, *versus* those obtained with OV-1701. As a preliminary
245 consideration, the results indicate that efficiency and inertness of both columns are very satisfactory, while
246 component recovery shows that the two SPs adsorb some components.

247

248 *Watercol™ 1460* – Table 2a reports the tailing factors (TF) and σ values of the components of the standard
249 mixture analyzed with this column. Cinnamyl alcohol (**4**), *p*-anisyl alcohol (**6**), vanillin (**9**), and α -pentyl
250 cinnamyl alcohol (**22**) were not detected, probably either because of irreversible adsorption, and/or of
251 retention outside the analysis time range (60 min), while phenylacetaldehyde (**2**) coeluted with neral (**11**),
252 and limonene (**5**), when injected in EtOH/H₂O, coeluted with water.

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

256 hydroxycitronellal (**17**) to 0.051 min for linalool (**14**). The high σ value (0.133 min) of limonene (**5**) was
257 probably due to its very low polarity, poorly compatible with the investigated SPs, together with its elution
258 within the solvent queue. Similar (and in some cases better) results were obtained with the same standard
259 mixture in EtOH/H₂O. Here, too, most peaks showed a tailing factor ranging between 0.9 and 1.1, but many
260 were perfectly symmetrical (TF 1.0), unlike the corresponding results with cyclohexane. The only exceptions
261 were again benzyl alcohol (**1**) (TF 1.2) and lylal a (**24a**) and b (**24b**), although their TF values fell to 1.2 and
262 1.3, respectively. Peak widths were in general in line with those of cyclohexane (sometimes slightly better)
263 and σ values ranged from 0.021 min with hydroxycitronellal (**17**) to 0.051 min for linalool (**14**).

264 Lastly, recovery percentage vs. OV-1701 was measured using benzyl benzoate (**25**) as internal reference for
265 normalization, its peaks with both 1460 and 1910 being of comparable intensity, as well as of similar
266 symmetry and width. Very few components gave a recovery below 65% with the standard mixture in
267 cyclohexane; those that did were hydroxycitronellal (**17**), eugenol (**16**), and farnesol isomers (**27**), whose
268 recoveries were 55%, 42% and 56% respectively (Figure 2a). The case of the sample in EtOH/H₂O was similar:
269 results were comparable or slightly better than in cyclohexane.

270

271 *WatercolTM 1910* – As reported elsewhere [11], the selectivity of 1910 differed from that of 1460. With 1910,
272 eugenol (**16**) and vanillin (**24**) were not detected, limonene (**5**) coeluted with both solvents (cyclohexane and
273 EtOH/H₂O), and the column failed to separate three pairs of compounds (i.e. phenylacetaldehyde (**2**)/ α -
274 ionone (**19**), estragole (**8**)/methyl octynoate (**12**), and hydroxycitronellal (**17**)/farnesol isomer b (**27b**)) (Table
275 2b).

276 Analysis of the standard mixture in cyclohexane also revealed good column performance. Most components
277 presented tailing factors between 0.8 and 1.2; the exceptions were neral (**11**) (TF 1.6) geraniol (**13**) (TF 1.9),
278 β -citronellol (**15**) (TF 1.9) and lylal isomer a (**24a**) (TF 1.3) and lylal isomer b (**24b**) (TF 1.5). Peak widths,
279 expressed as σ values, ranged from 0.023 min for α -pentyl cinnamyl alcohol (**22**) and benzyl benzoate (**25**) to
280 0.044 min for geranial (**10**), the sole exception being lilial (**21**) whose σ was 0.093 min.

281 The same standard mixture solubilized in EtOH/H₂O and analyzed with 1910 column gave better results for
282 most components. Most peaks were characterized by tailing factors ranging from 0.9 to 1.1, and some were
283 perfectly symmetrical (TF 1.0). TF was equal or better for all components, baring neral (**11**), which had a
284 higher TF (1.9) than in cyclohexane. Peak widths, expressed as σ values, ranged from 0.024 min for methyl
285 eugenol (**18**) to 0.055 min for geranial (**10**), again with the exception of lilial (**21**) (0.111 min).

286 Again for 1910, percentage recovery vs. OV-1701 was measured using benzyl benzoate (**25**) as internal
287 reference (Figure 2b). This column provided recoveries above 65% for all components with the standard
288 mixture in cyclohexane, with the exception of lylal isomer a (**24a**) (25%), lylal isomer b (**24b**) (28%), farnesol
289 isomer a (**27a**) (59%), and benzyl cinnamate (**29**) (53%). The standard mixture in EtOH/H₂O gave recoveries

290 below 65% only for neral (**11**) (62%), benzyl cinnamate (**29**) (53%), and lylal isomers a (**24a**) (29%) and b (**24b**)
291 (37%).

292

293 3.1.2 Tuning the injection modality

294 The previous paragraph showed that both 1460 and 1910 columns performed correctly on the reference test
295 mixture, with either cyclohexane or EtOH/H₂O as solvent. The main drawback was the rather poor
296 repeatability with the EtOH/H₂O mixture, compared to that with cyclohexane (Table 3). The results indicate
297 that the cause of this inconsistency is injection. Repeatable and non-discriminative split injection, in particular
298 for quantitative analysis, requires the correct transfer of the sample, fully vaporized, into the injector body
299 at the head of the column, where a narrow band must be generated. This step not only entails selecting the
300 correct temperature, but also applying a suitable injector head pressure that is compatible with both the
301 nature of the solvent and the liner volume and shape [18, 19]. When applied to aqueous samples, the
302 injection conditions adopted with conventional solvents can lead to non-homogeneous sample transfer at
303 the head of the column. This is because of the physico-chemical characteristics of water, which, under the
304 same conditions used for conventional solvents, requires more heat and a longer time to vaporize, produces
305 a large volume of steam and, importantly, slows the transfer to the head of the column because of its
306 viscosity. For instance, 1.0 μL of water in the liquid state, injected at 200 kPa at 200°C, produces a volume of
307 steam of 722 μL , while at 230°C the volume is 768 μL , whereas, under the same conditions, cyclohexane gives
308 120 μL and 128 μL , respectively [20]. The volume of a conventional liner with a 5 mm plug of silanized glass
309 wool is about 800 μL , i.e. very close to the volume of steam at the two temperatures, while that of
310 cyclohexane is within the optimal range for correct transfer.

311 These large steam volumes can induce randomized sample and analyte discrimination, and irregular transfer
312 to the column, in particular affecting the quantitative performance of the injection step. Injection
313 performance can be improved by acting on both the steam volume at the applied temperature and the liner
314 characteristics. The volume can be reduced by increasing the pressure inside the injector, i.e. by operating in
315 an over-pressurized split injection mode. The volume of water vapor phase produced at 350 kPa at 200°C in
316 the injector body falls to 482 μL , thus providing better compatibility with the liner volume. In this study, most
317 samples were solubilized in EtOH/H₂O, giving a final vapor nominal volume of 353 μL at 350 kPa and 200°C,
318 and of 377 μL at 230°C calculated for each solvent with the dedicated Agilent calculator and mediated for
319 the solvent mixture [20]. The liner was also adapted to the volume of steam, which was in any case above
320 that of conventional solvents (e.g. cyclohexane), by including a double layer of silanized glass wool plug (i.e.
321 ca 1 cm). Injection pressure of 350 kPa was chosen as it gave the best results in a series of experiments carried
322 out with pressure ranging from 250 to 450 kPa. This pressure, applied to the 1460 and 1910 columns, with
323 hydrogen as carrier gas, led to a very marked improvement of repeatability of the components of the allergen
324 test mixture in EtOH/H₂O, making correct quantitative analysis possible (Table 3). The standard mixture in

325 cyclohexane, injected in 1460 under conventional injection conditions (200°C/200 kPa), presented RSD%
326 calculated on six replicates ranging from 0.2% for benzyl salicylate (**28**) and benzyl cinnamate (**29**) to 6.8%
327 for hydroxycitronellal (**17**). The same mixture in these conditions in EtOH/H₂O ranged from 11.9% for
328 estragole (**8**) to 52.8% for lylal isomer a (**24a**) whereas when injected at high pressure (230°C/350 kPa), it
329 gave RSD% ranging from 3.6% for α-ionone (**19**) and amyl cinnamaldehyde (**20**) to 6.2% for α-isomethyl
330 ionone (**23**). Similar results were obtained with 1910 with the same standard mixture at 230°C/200 kPa; in
331 cyclohexane, RSD% ranged from 1.0% for cinnamaldehyde (**3**) to 10.8% for benzyl cinnamate (**29**). In
332 EtOH/H₂O, it ranged from 6.3% for cinnamaldehyde (**3**) to 38.0% for lylal isomer b (**24b**). The RSD% of the
333 same sample in EtOH/H₂O, but at 200°C/350 kPa, ranged from 0.2% for linalool (**14**) and β-citronellol (**15**) to
334 5.5 % for lylal isomer b (**24b**).

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

340 The high efficiency and selectivity of both the Watercol™ columns investigated assure good results, even
341 with the usual 10 m NB columns, which required the applied helium pressures to be reduced to values
342 comparable to those previously optimized, i.e. 240 kPa at the head of the column, with over-pressurization
343 at 350 kPa for one minute during injection. The results indicated a maximum RSD% of 9.4% for benzyl
344 salicylate (**28**) with 1460, and of 6.6% for neral (**11**) with 1910. The difference of repeatability between GC-
345 MS and GC-FID, although always acceptable, are probably justified by the different nature of the gas carrier
346 and overpressure/pressure ratio. For both columns, the EtOH/H₂O results were often better than those with
347 cyclohexane as main solvent.

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

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

356

357 3.1.3 Mass spectrometry and water injection

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

361

362 3.2 Direct quantitation of suspected allergens and markers in commercial perfumes

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

370 The following suspected allergens were identified: *perfume 1*: benzyl alcohol (**1**), cinnamyl alcohol (**4**),
371 coumarin (**9**); geraniol (**13**), linalool (**14**), β -citronellol (**15**), eugenol (**16**), hydroxycitronellal (**17**), lilial (**21**), α -
372 isomethyl ionone (**23**), lylal isomer a (**24a**), lylal isomer b (**24b**), hexyl-cinnamaldehyde (**26**), linalyl acetate
373 (**30**); *perfume 2*: geraniol (**13**), linalool (**14**), β -citronellol (**15**), and linalyl acetate (**30**). Although not on the
374 original list of allergens, linalyl acetate (**30**) was also studied, since it was included in the 2011
375 recommendation of the Scientific Committee on Consumer Safety, containing 54 compounds and isomers
376 and some extracts [4].

377 Table 1 reports the figures of merit of the quantitative method of the investigated allergens for both
378 Watercol™ columns; they are representative of the method's analytical performance. All compounds were
379 quantified by external calibration with pure standards, using carvacrol as internal standard. The linearity of
380 the method for all analytes was investigated over a wide range of concentrations (5-1000 mg/L) chosen in
381 consideration of the widely differing compositions of commercial perfumes, as is indeed shown by the two
382 investigated. In spite of the very wide concentration range considered, linearity, measured in terms of
383 regression coefficients (R^2), was very good for all analytes with both columns, the minimum R^2 in all cases
384 being above 0.9917 (linalyl acetate (**30**)) for 1460, and 0.9969 (lilial (**21**) and linalyl acetate (**30**)) for 1910.
385 Similar consideration can be made for area repeatability: the maximum RSD% values (n=6) were 7.5% for
386 lylal isomer b (**24b**) for 1460, and 7.3% for lylal isomer a (**24a**) with 1910; likewise, maximum intermediate
387 precision was 12.4% for geraniol (**13**) for 1460, and 11.9% for lylal isomer a (**24a**) with 1910. The high values
388 for lylal isomers may partly be due to their tailed peak shape, which might interfere with correct area
389 determination. With both Watercol™ columns, LODs and LOQs of all components of the perfumes, diluted
390 1:10, were in conformity with the leave-on allergen declaration (0.01%). LODs ranged between 1.1 mg/L for
391 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
392 hydroxycitronellal (**17**) for 1910. LOQs were between 3.1 mg/L for hexyl cinnamaldehyde (**26**) and 7.3 mg/L

393 for lylal isomer a (**24a**) with 1460, and 3.2 mg/L for coumarin (**7**) and 7.2 mg/L again for lylal isomer a (**24a**)
394 with 1910. Table 4 reports quantitative data and repeatability of the allergens identified in the two
395 commercial perfumes investigated, by direct injection in GC-MS with both Watercol™ columns; *perfume 1*
396 was diluted 1:10 and *perfume 2* 1:20, so as to fall within the range of linearity (5-1000mg/L) of linalyl acetate
397 (**30**). The results with the two columns were highly consistent, the percent result differences for all analytes
398 being in all cases below 9%, with the exception of lylal isomer a (**24a**) (11.2%). These results were confirmed
399 by their full agreement with those obtained on the same perfumes analyzed with the reference IFRA method
400 (data not reported).

401

402

403 **4. Conclusions**

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

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

427

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432 **Compliance with ethical standards**

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

434

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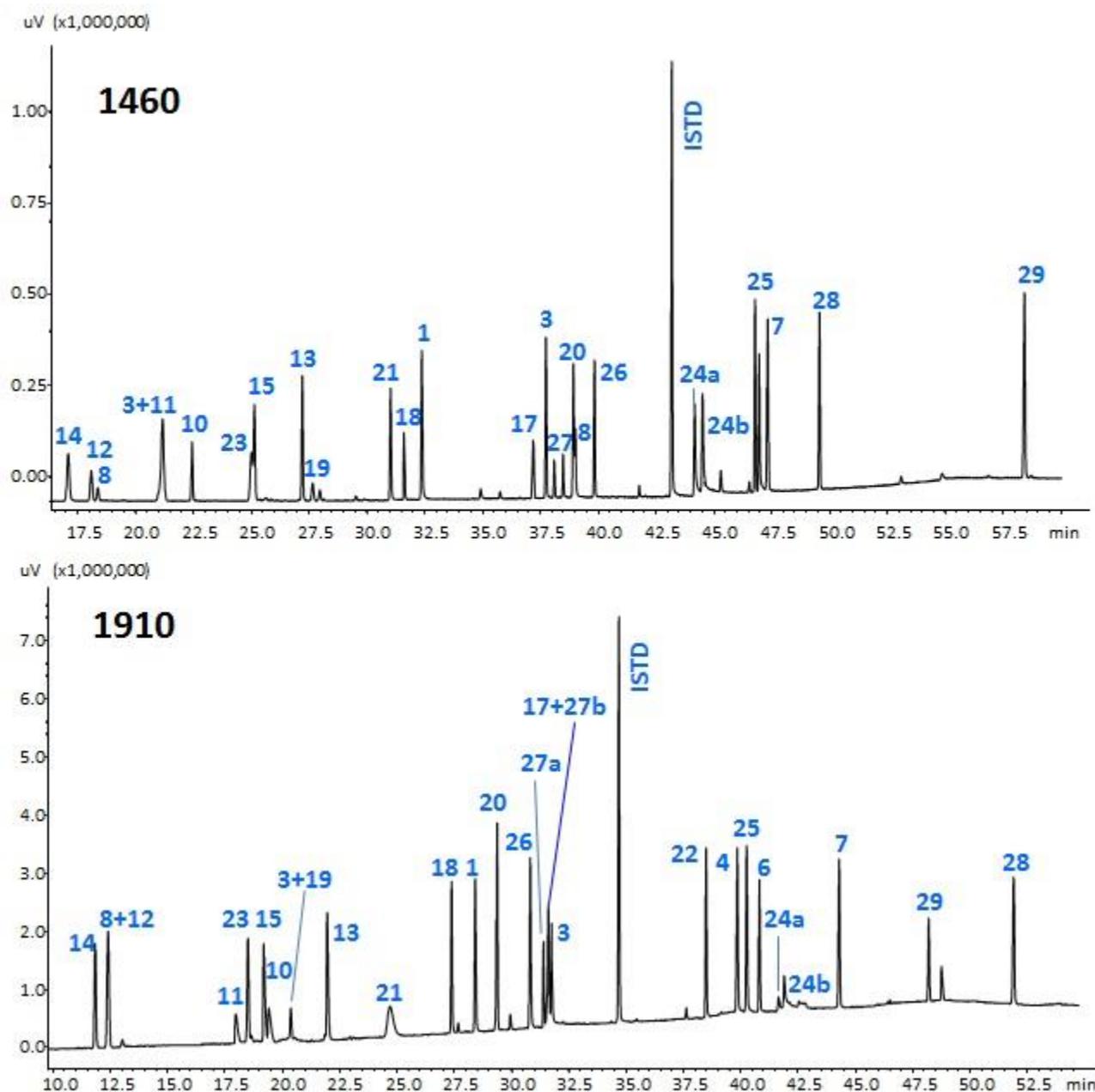
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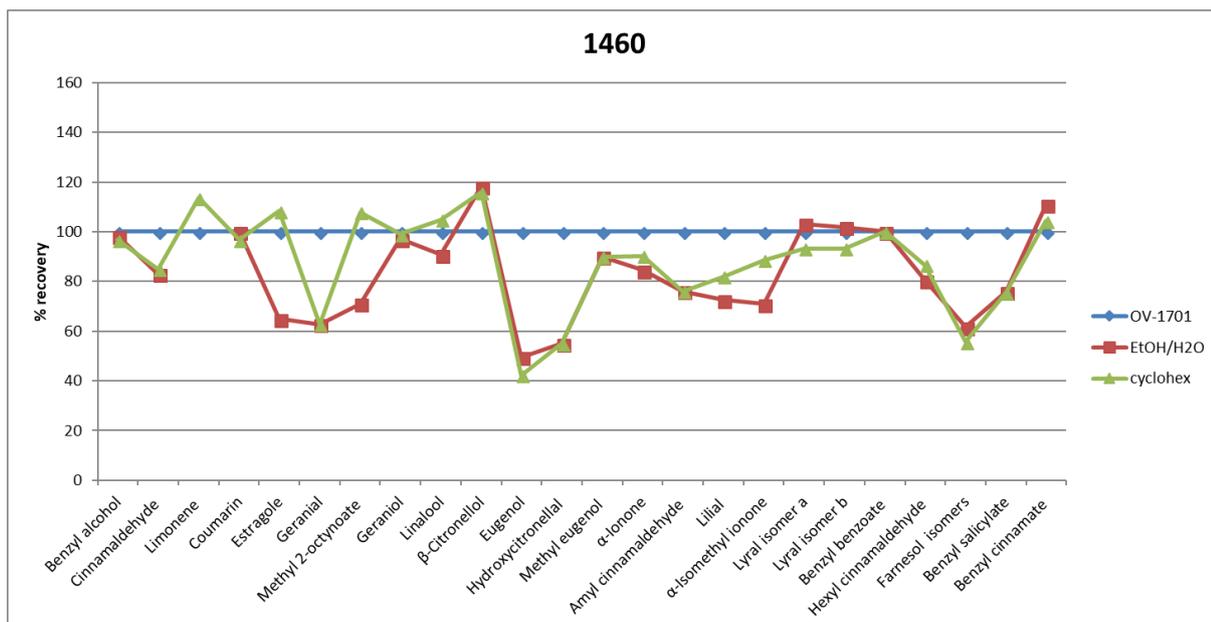
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489 **Figure 1** - GC-FID patterns of the allergen standard mixture diluted in 1:1 EtOH/H₂O analyzed with Watercol™
 490 1460, and 1910. For analysis conditions see text. Peak identification: **1.** benzyl alcohol, **2.**
 491 phenylacetaldehyde, **3.** cinnamaldehyde, **4.** cinnamyl alcohol, **5.** limonene, **6.** anisyl alcohol, **7.** coumarin, **8.**
 492 estragole, **9.** vanillin, **10.** geranial, **11.** neral, **12.** methyl-2-octynoate, **13.** geraniol, **14.** linalool, **15.** β-
 493 citronellol, **16.** eugenol, **17.** hydroxycitronellal; **18.** methyl eugenol, **19.** α-ionone, **20.** amyl cinnamaldehyde,
 494 **21.** lilial, **22.** α-pentyl cinnamyl alcohol, **23.** α-isomethyl ionone, **24a.** lyral isomer a, **24b.** lyral isomer b, **25.**
 495 benzyl benzoate, **26.** hexyl cinnamaldehyde, **27.** farnesol isomers, **28.** benzyl salycilate, and **29.** benzyl
 496 cinnamate.

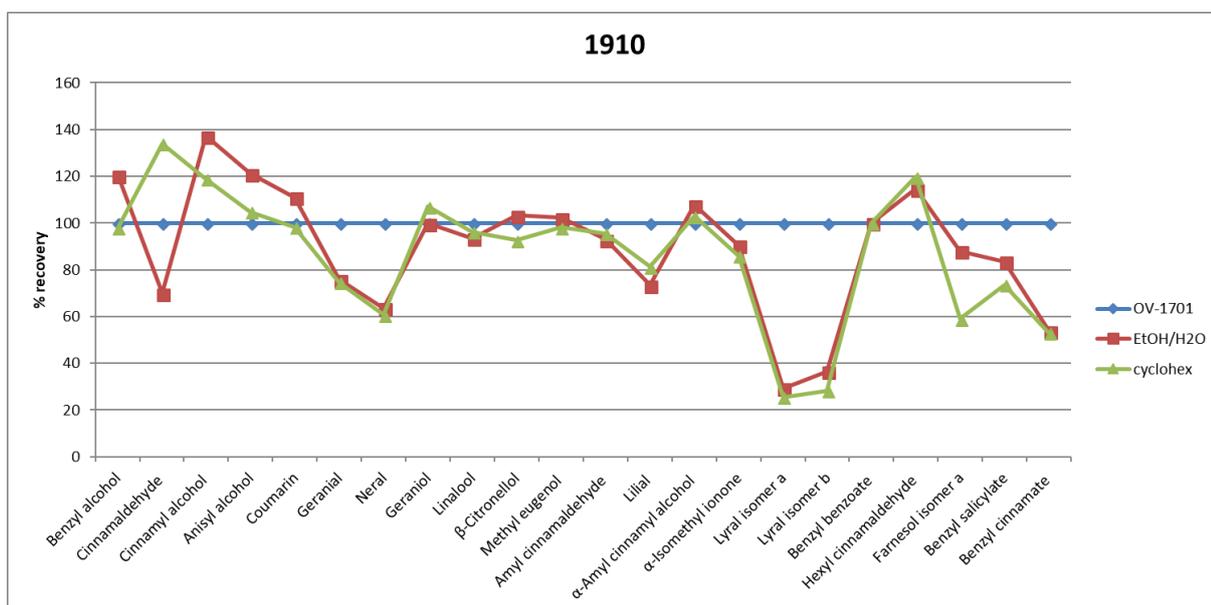
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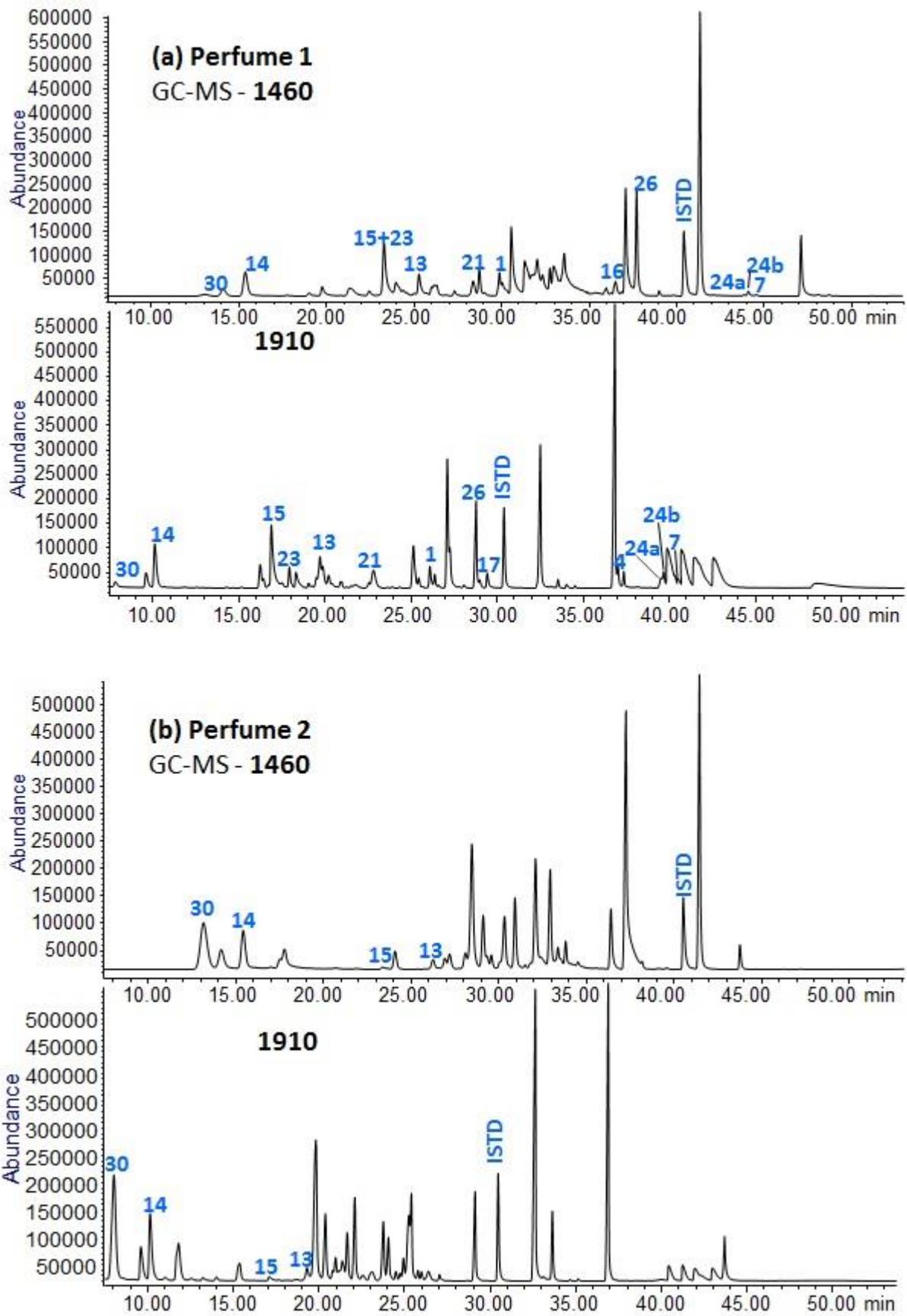
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501 **Figure 2** - Recovery of suspected allergens, calculated from the normalized absolute area of each analyte,
502 with Watercol™ 1460 and 1910, versus OV-1701 columns, taken as reference.



503

504

505

Figure 3 - GC-MS patterns of perfume 1 (a) and perfume 2 (b) on Watercol™ 1460 and 1910. For analysis conditions see text. Peak identification: **1.** benzyl alcohol, **4.** cinnamyl alcohol, **7.** coumarin, **13.** geraniol, **14.**

506 linalool, **15.** β -citronellol, **16.** eugenol, **17.** hydroxycitronellal, **21.** linal, **23.** α -isomethyl ionone, **24a.** and **24b.**
507 linal isomers, **26.** hexyl cinnamaldehyde and **30.** linalyl acetate
508
509
510
511

512 Captions to tables

513

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

N	Compound	Selected ions	1460					1910				
			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	Lyr al 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	Lyr al 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

515 linearity, R²: regression coefficient; LOD: limit of detection; LOQ: limit of quantitation; ND: not detected; Rep.:

516 repeatability, calculated at 50 mg L⁻¹ (n= 6); I.P.: Intermediate Precision calculated at 50 mg L⁻¹

517

518 **Table2** - Figures of merit (retention time, tailing factor and σ of the peak width) of the two Watercol™

519 columns investigated, calculated from the analysis of the allergen standard mixture in cyclohexane and

520 EtOH/H₂O with; ND: not detected, NM: not measurable (partial coelutions); a) 1460 NB column 15 m,

521 detector FID, carrier: H₂; coelutions: *coel 1*: phenylacetaldehyde (**2**)/neral (**11**); b) 1910 NB column 15 m,

522 detector FID, carrier: H₂; coelutions: *coel 2*: phenylacetaldehyde (**2**)/α-ionone (**19**); *coel 3*: estragole

523 (**8**)/methyl octynoate (**12**); *coel 4*: hydroxycitronellal (**17**)/farnesol isomer a (**27a**)

524

2a	1460	Cyclohexane		EtOH/H₂O 1:1
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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	<i>coel 1</i>	<i>coel1</i>		21.12	<i>coel 1</i>	<i>coel 1</i>
3	Cinnamaldehyde	37.92	1.0	0.023		37.90	1.0	0.025
4	Cinnamyl alcohol	<i>ND</i>	<i>ND</i>	<i>ND</i>		<i>ND</i>	<i>ND</i>	<i>ND</i>
5	Limonene	3.09	1.1	0.133		<i>NM</i>	<i>NM</i>	<i>NM</i>
6	Anisyl alcohol	<i>ND</i>	<i>ND</i>	<i>ND</i>		<i>ND</i>	<i>ND</i>	<i>ND</i>
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	<i>ND</i>	<i>ND</i>	<i>ND</i>		<i>ND</i>	<i>ND</i>	<i>ND</i>
10	Geranial	22.57	0.9	0.040		22.56	0.9	0.037
11	Neral	21.14	<i>coel 1</i>	<i>coel 1</i>		21.12	<i>coel 1</i>	<i>coel 1</i>
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	<i>NM</i>	<i>NM</i>		25.26	<i>NM</i>	<i>NM</i>
16	Eugenol	39.21	<i>NM</i>	<i>NM</i>		39.17	<i>NM</i>	<i>NM</i>
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	<i>NM</i>	<i>NM</i>		38.09	<i>NM</i>	<i>NM</i>
21	Lilial	31.18	1.0	0.025		31.16	1.1	0.026
22	α -Pentyl cinnamyl alcohol	<i>ND</i>	<i>ND</i>	<i>ND</i>		<i>ND</i>	<i>ND</i>	<i>ND</i>
23	α -Isomethyl Ionone	25.16	<i>NM</i>	<i>NM</i>		25.13	<i>NM</i>	<i>NM</i>
24a	Lylal isomer a	44.37	2.0	0.043		44.35	1.2	0.028
24b	Lylal 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	Farnesol isomer a	38.67	1.1	0.22		38.64	1.1	0.023
27b	Farnesol isomer b							
28	Benzyl salicylate	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	<i>coel 2</i>	<i>coel 2</i>		20.56	<i>coel 2</i>	<i>coel 2</i>
3	Cinnamaldehyde	31.82	<i>NM</i>	0.040		31.96	<i>NM</i>	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	Lyr al isomer a	41.86	1.3	0.029		41.88	1.2	0.029
24b	Lyr al 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 salicylate	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

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527 **Table 3** - GC-FID and GC-MS injection repeatability (RSD%) of the Watercol™ columns, calculated through
528 analysis of the allergen standard mixture (n=6). Solvents: cyC₆: cyclohexane, Et-H₂O: ethanol/water 1:1; CI:
529 conventional injection, OpIn: overpressurized injection (H₂: 350 kPa, He: 350 kPa); ND: not detected, NM: not
530 measurable (co-elutions)

RSD%	GC-FID						GC-MS			
	col. length: 15 m; carrier gas: H ₂						col. length: 10 m; carrier gas: He			
	1460			1910			1460		1910	
Compounds	cyC ₆	EtOH/H ₂ O		cyC ₆	EtOH/H ₂ O		cyC ₆	EtOH/H ₂ O	cyC ₆	EtOH/H ₂ O
	CI	CI	OpIn	CI	CI	OpIn	CI	OpIn	CI	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	Lyr al isomer a	6.0	52.8	4.6	4.1	28.9	4.5	9.1	7.5	2.9	5.9
24b	Lyr al 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

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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 %: $(Q_{1910} - Q_{1460}/Q_{1910})\%$

Perf	ID	Analyte	1910			1460			1910/1460
			Abs. am. mg/L (ppm)	SD	RSD%	Abs. am. mg/L (ppm)	SD	RSD%	Δ %
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>i</i> -Methyl ionone	485	4	0.9	527	48	9.0	7.9
	24a	Lyr al isomer a	1624	32	2.0	1492	102	7.3	8.9
	24b	Lyr al 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

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