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Human odor and forensics. Optimization of a comprehensive two-dimensional gas chromatography method based on orthogonality: how not to choose between criteria.

Authors: Vincent Cuzuel ^{a,b}, Audrey Sizun ^b, Guillaume Cognon ^b, Isabelle Rivals ^c, François Heulard ^b, Didier Thiébaud ^a, Jérôme Vial ^a

Corresponding author: Vincent Cuzuel, vincent.cuzuel@espci.fr, +33140794644

^a UMR CBI 8231, CNRS, Laboratoire Sciences Analytiques Bioanalytiques et Miniaturisation, ESPCI Paris, PSL Research University, 10 rue Vauquelin, PARIS, France

^b Institut de Recherche Criminelle de la Gendarmerie Nationale, Caserne Lange, 5 Boulevard de l'Hautil, BP 20312 PONTOISE, 95037 CERGY PONTOISE CEDEX, France

^c Equipe de Statistique Appliquée, ESPCI Paris, PSL Research University, INSERM, UMRS 1158 Neurophysiologie Respiratoire Expérimentale et Clinique, 10 rue Vauquelin, PARIS, France

Abstract:

The use of comprehensive two-dimensional gas chromatography coupled with mass spectrometry would be a real asset for the forensic profiling of human hand odor. This paper focuses on the optimization of a comprehensive gas chromatography method using a synthetic mixture of 80 compounds representative of human hand odor composition. In order to rank the candidate column sets, instead of using a unique criterion, we used a chemometric tool called desirability which is based on Derringer functions and enables to consider several criteria simultaneously and hence to get the best compromise. Nine criteria including six orthogonality criteria were used to evaluate the quality and the efficiency of the separation. The desirability analysis lead to a straightforward ranking and an accurate overview of the results in two situations, with an objective of routine analysis and without. In both cases, the DB-1MS×DB-1701 set was found to be best suited for the separation of the considered mixture, however with different gradients.

Keywords:

Comprehensive two-dimensional gas chromatography; orthogonality; desirability analysis; forensic; human hand odor

1) Introduction

Profiling human hand odor is a topic of current interest because it would be a considerable breakthrough in forensics, bringing more convincing evidence to magistrates than that of dogs alone. To give more relevance to the information obtained from dogs, there is a need to develop a better understanding of human odor. Indeed, relying only on animals whatever their capabilities is not suitable in courts: identification provided by dogs can merely be used as a corroborative evidence. Thus, the development of an objective analytical strategy to

37 characterize human odor may be a promising complementary tool to the use of trained dogs
38 [1,2]. Hundreds of molecules are involved in human hand odor. To our knowledge however,
39 two-dimensional (2D) techniques have not yet been used to characterize living human odor
40 though comprehensive two-dimensional gas chromatography coupled with mass
41 spectrometry (GC×GC-MS) may provide novel information regarding the complexity of the
42 samples.

43

44 Like fingerprints, human odor is said to be specific of each individual [2]. In 2014, de Lacy
45 Costello *et al.* [3] published a comprehensive review of the volatiles emanating from a healthy
46 human body. Different groups of chemical compounds were found in the headspace of
47 biological samples: acids, alcohols, aldehydes, esters, hydrocarbons, ketones, heterocyclic
48 compounds [4] and even sulfur-containing compounds in armpit samples [5]. Considering the
49 nature of the molecules involved, gas chromatography coupled with mass spectrometry (GC-
50 MS) seems to be the most appropriate analytical technique [6]. However, classic one-
51 dimensional (1D) GC-MS showed its limitations in term of peak capacity considering the
52 number of compounds involved: using GC×GC-MS appeared natural to overcome the
53 complexity of the samples.

54 The first thing to consider when developing a 2D method is the choice of the column set, and
55 orthogonality is often used to characterize the performance of a 2D separation. A system is
56 said to be orthogonal if the first and second dimensions columns are statistically independent.
57 But the independence of the two retention mechanisms might not be sufficient to provide a
58 better separation. Indeed, any change of selectivity between first and second dimension
59 provides always a better separation than that obtained in 1D, but for an efficient separation,
60 the surface coverage of the 2D chromatogram by the spots (compounds) also has to be
61 homogenous. The evaluation of the quality of the separation is still in the center of many
62 debates and there are many criteria for its evaluation, some of them characterizing the
63 absence of correlation or independence, others the occupation of the separation space, and
64 still others trying to combine the two. Schure and Davis recently compared several criteria
65 used for the evaluation of 2D separations [7]: the choice of a criterion appears to be tough,
66 each of them having its advantages and limitations. Though this paper aims neither at
67 reviewing all criteria described in the literature, nor at comparing their performance, we
68 propose here a short overview of the most representative ones.

69 Correlation coefficients (Pearson's, Kendall's and Spearman's) were used to evaluate the
70 correlation between the retention times of the two dimensions of a 2D separation [8–11]. Al
71 Bakain *et al.* showed that Kendall's coefficient was a good indicator of orthogonality: contrary
72 to Pearson's coefficient, Kendall's is not sensitive to extreme values and provides a good
73 measure of nonlinear monotonous association [8]. However, Gilar *et al.* stated that correlation
74 coefficients were not well suited for measurement of chromatographic orthogonality,
75 especially for systems with clustered data and outliers [12]. Slonecker *et al.* proposed two
76 criteria based on informational theory, the informational similarity and the percentage of
77 synentropy, both based on the mutual information of the system [13], which is a measure of
78 independence.

79 Other descriptors rather characterize the distribution of the peaks in the 2D space, using for
80 example nearest neighbor distances (NND) between peaks. One can either sum distances, as
81 discussed by Clark and Evans [14], or squared distances, as discussed by Brown and Rothery
82 [15]. Nowik et al. also used a NND approach in their work [16,17]; the arithmetic mean of the
83 NND gives a good indication of peak spreading all over separation space, but is strongly
84 impacted by long distances. The authors also used the geometric and harmonic means which
85 are descriptors of homogeneity and are also sensitive to short distances. They concluded that
86 the harmonic mean of the NND best characterizes orthogonality. Schure et al. proposed a
87 measure of retention time spacing based on a power law fractal distribution [18]. The box-
88 counting dimension is a measure of inter-peak spacing scales and, indirectly, of how effectively
89 the separation space is used [12,18]. The homogeneity of the surface coverage can also be
90 more directly characterized by discretizing the 2D space in bins and evaluating the ratio
91 between number of occupied bins and total number of bins [8,12,19]. This method can be
92 applied either on the original retention times, or on values normalized between the smallest
93 and the largest one: in the latter case, the unused separation space on the borders is not taken
94 into account. Leonhardt et al. compared the compound distribution in each dimension to the
95 distribution of a fully orthogonal system to evaluate clustering [20]. Ryan et al. measure
96 directly the ratio between the area occupied by the spots and the unused separation space
97 beneath the second dimension [21]. The area of occupied space can also be estimated by a
98 rectangular form. However, Semard et al. pointed out that a rectangle is too coarse to describe
99 a complex geometry, and proposed to estimate the percentage of occupied separation space
100 using Delaunay's triangulation algorithms based on the convex hull [22]. Rutan et al. also used
101 the minimum convex hull to determine a fractional coverage metrics characterizing the
102 effective peak capacity [23]. Another geometric approach consists in the calculation of the
103 peak spreading angle [11,24]. Gilar et al. concluded that surface coverage and box-counting
104 dimension methods offer useful and intuitively understandable measures of orthogonality.
105 These methods are robust enough for small samples commonly used in chromatographic
106 practice [12].

107 In order to take both independence of the retention times and coverage of the separation
108 space into account, Zeng et al. proposed a criterion based both on the bin coverage
109 percentage and Pearson's linear correlation coefficient between the retention times [25]. In
110 order to account for a large variety of nonlinear relationships, Mani-Varnosfaderani et al.
111 adapted this method by replacing Pearson's correlation by the maximal information
112 coefficient [26]. Jacova et al. evaluated different orthogonality assessment tools in GC×GC
113 [27], and using simulation and statistics, they concluded that the geometric approach using a
114 number of bins equal to the number of compounds [8,12] or the NND approach with the use
115 of the arithmetic and the harmonic means [16,17] were the best ones.

116 This short survey shows the extreme diversity of the criteria for the evaluation of 2D
117 separations: considering a single criterion is not an option anymore [28]. This is the reason
118 why, in this paper, nine criteria including six orthogonality criteria were considered for the
119 evaluation of the separation of a synthetic mixture of 80 representative of human hand odor
120 [2]. The criteria were selected for the complementarity of the information they provide. A
121 chemometric tool called desirability [29,30] using Derringer functions was used to get an
122 accurate overview of results [31]. Its optimization led to the best compromise between the

123 criteria and provided an overall quality score, enabling to rank the different column sets
124 objectively.

125 II) Statistical analysis

126 1) Criteria

127 Six orthogonality criteria were selected: Spearman's correlation coefficient, the arithmetic
128 and the harmonic means of the NND, the coefficient of orthogonality calculated with the
129 Camenzuli's Asterisk equations [32], and the calculation of the separation space coverage with
130 both normalized and raw data. The quality of the separation was also evaluated with three
131 additional criteria: the number of coelutions, the number of detected compounds and the run
132 time. The latter criteria and the reason why they were chosen will be detailed in the present
133 section.

134 Let n be the number of detected compounds of the chromatogram, and 1t and 2t the
135 retention times of the first and second dimension respectively. Let ${}_{min}^1t$, ${}_{max}^1t$, ${}_{min}^2t$ and ${}_{max}^2t$
136 be the retention times of the first and the last eluted compounds in each dimension. The
137 normalized retention times of the first and second dimension ${}_{norm}^1t$ and ${}_{norm}^2t$ are calculated
138 as follows:

$$139 \quad {}_{norm}^i t = \frac{{}^i t - {}_{min}^i t}{{}_{max}^i t - {}_{min}^i t} \quad (1).$$

140

141 1.1 Spearman's correlation coefficient

142 If the peaks are well-spread all over the 2D separation space, the first and second dimension
143 retention times should not be correlated. Thus, correlation coefficients can be used to
144 evaluate orthogonality [8], with the advantage that they do not require the discretization of
145 the separation space, nor the normalization of the retention times, and can be calculated
146 easily.

147 Spearman's correlation coefficient ρ provides a measure of monotonous nonlinear
148 correlations between retention times. The compounds are ranked according to their retention
149 time in the first and second dimensions. Let (r_i^{t1}, r_i^{t2}) denote the ranks of a pair of coordinates
150 of compound i , $d_i = r_i^{t1} - r_i^{t2}$, and n the number of compounds, then:

$$151 \quad \rho = 1 - \frac{6 \sum_{i=1}^n d_i^2}{n(n^2 - 1)} \quad (2)$$

152 Spearman's correlation coefficient is preferred to Pearson's which is not suited for the
153 measure of nonlinear associations and is sensitive to extreme values.

154

155 1.2 Nearest Neighbor distance approach

156 A recent approach was proposed by Nowik *et al.* to assess the orthogonality of 2D separative
157 systems using NND [16]. The NND is defined as the shortest of all distances l_i of point i to all
158 other points. All calculations were performed with normalized retention time, so that only the

159 time window effectively occupied by the compounds, which is usually narrower than the
160 whole separation space, is considered. Three different descriptors were defined by Nowik *et*
161 *al.*: the arithmetic mean $\bar{A}_{(o)}$, the geometric mean $\bar{G}_{(o)}$ and the harmonic mean $\bar{H}_{(o)}$.

$$162 \quad \bar{A}_{(o)} = \frac{\sum_{i=1}^{n-1} l_i}{n-1} \quad (3) \quad \bar{G}_{(o)} = \left(\prod_{i=1}^{n-1} l_i \right)^{\frac{1}{n-1}} \quad (4) \quad \bar{H}_{(o)} = \frac{n-1}{\sum_{i=1}^{n-1} \frac{1}{l_i}} \quad (5)$$

163 The arithmetic and the geometric means are strongly influenced by long distances and give
164 information about peak spreading, while the harmonic mean is more impacted by short
165 distances and therefore gives information about clustering.

166

167 1.3 Asterisk equations

168 Camenzuli *et al.* proposed a new measure of orthogonality for multi-dimensional
169 chromatography [32]. Different equations – known as Asterisk equations – allowed the
170 calculation of A_0 which gave a measure of orthogonality. All calculations were performed with
171 normalized retention times. Four parameters are calculated to characterize the peak
172 dispersion around four lines. The peak dispersion around each line is compared to that of a
173 uniform distribution. This technique does not require the discretization of the separation
174 space and has the advantage of evaluating the dispersion around four different axes.

175

176 1.4 Occupied space – geometric criteria

177 This geometric criterion was used as an indicator of the coverage of the separation space and
178 hence of orthogonality [8]. It is impacted by the number of peaks and requires space
179 discretization into $p \times p$ bins. In the present study, the mixture of standards contains 80
180 compounds. A value of $p=9$ was chosen to have a number of bins close to the number of
181 targeted compounds: one compound per bin would lead to a space covering percentage of
182 98.7%.

183 This geometric criterion can be calculated using either raw or normalized data. Calculation
184 with normalized data eliminates border areas of the chromatogram, and enables to consider
185 only the relative spatial arrangement of the compounds, whereas working with raw data
186 enables to take the undesired presence of empty areas into account.

187

188 1.5 Additional separation criteria

189 Three additional criteria were included for the optimization of the columns set and conditions.
190 Since the developed methods will eventually be used routinely, the run time was taken into
191 account to save time. The number of coelutions as well as the number of detected compounds
192 – compounds of the mixture found on the considered chromatogram - were also evaluated to
193 rank the different columns set. Plotting the number of coelutions versus the number of
194 detected compounds for each analytical setup showed that these two indicators are
195 absolutely not correlated (Pearson correlation coefficient of 6.10^{-5}). Indeed, the modulation
196 system being a dual-jet cryomodulator applied to the second dimension column – on which
197 the modulation is done - some compounds can be subject to an echo phenomenon. A cold-jet

198 default of trapping can lead to the apparition of twin peaks separated by half a modulation
199 time in the second dimension. Thus, an increase in the number of coelutions can be observed
200 while the number of detected compounds remains the same.

201

202 2) Desirability parameters

203 Desirability evaluation is a multicriteria decision-making method using Derringer functions
204 [31], which necessitates the definition of an individual desirability function for each criterion.
205 In this way, each measured response of interest is converted into a dimensionless number d_k
206 between 0 (criterion not satisfied) and 1 (criterion satisfied). The two bounds of the
207 desirability curve will be described by the letters B_0 and B_1 (cf. figure 1).

208 The global desirability D of a column set is calculated as the product of the individual
209 desirabilities:

210

211

$$D = \prod_k d_k$$

212 The desirability approach allows to get an accurate overview of results in a straightforward
213 manner, as in [30,33,34] where it was successfully applied for multicriteria optimization.

214 3) Software

215 MATLAB (Mathworks, Natick, MA, USA) software v. 9.0.0.341360 (2016a) was used to process
216 the data.

217

218 III) Material and methods

219

219 1) Chemicals and reagents

220 To optimize the analytical methods, a mixture of 80 standards was prepared in heptane (10
221 mg/kg each). The detailed composition of this mixture is available as Supplementary Material.
222 Compounds likely to be found in human hand odor were selected [2]. A particular attention
223 was given to cover a wide range of chemical properties (polarity, molecular weight, chemical
224 family).

225 Individual standards of the 80 targeted compounds were purchased from Sigma-Aldrich (Saint
226 Quentin Fallavier, France). Methanol was purchased from Carlo Erba Reagents (Fontenay-
227 sous-bois, France). Ultra-pure water was produced using a Direct-Q UV 3 system (18.2 MΩ/cm)
228 from Millipore (Molsheim, France).

229

230 2) Comprehensive two-dimensional gas chromatography and mass 231 spectrometry

232 Analyses were performed on a Thermo Trace GC×GC coupled with a Thermo ISQ MSD
233 (Thermo, Villebon-sur-Yvette, France). This device was equipped with a Thermo Triplus
234 autosampler.

235 Helium flow was set at a constant value of 1mL/min. The initial temperature was 40
236 °C, held for 1 min, then raised to 250 °C, and again held for 1 min, with gradients of 2, 2.5, 3
237 or 4 °C/min. The modulation was performed with a dual-jet CO₂ thermal modulator, and
238 modulation times of 6, 8 or 10 seconds were selected. The mass spectrometer was used with
239 the electronic ionization source (70 eV) heated at 210°C. The acquisition was made in scan
240 mode, with scan range 46-320 amu. A solvent delay of 9 minutes was used. Data were
241 acquired with Xcalibur and processed with ChromCard (Thermo softwares).

242

243 3) Columns

244 Several first dimension columns (30 m x 0.25 mm, 0.25 µm) were tested and purchased from
245 Agilent (Les Ulis, France) to conduct the first chromatographic separation: 100%
246 Dimethylpolysiloxane (DB-1MS), (5%-Phenyl)-methylpolysiloxane (DB-5MS), (14%-
247 Cyanopropyl-phenyl)-methylpolysiloxane (DB-1701), Polyethylene glycol (DB-WAX).

248 They were coupled with the following second dimension columns (1.5 m x 0.10 mm, 0.10 µm):
249 DB-1, DB-5, DB-1701, DB-WAX columns purchased from Agilent (Les Ulis, France) and a non-
250 bonded 1,12-Di(triisopropylphosphonium)dodecane bis(trifluoromethanesulfonyl)imide phase
251 (SLB-IL60, 1m x 0.10 mm, 0.08 µm) purchased from Supelco (Sigma–Aldrich, Saint Quentin
252 Fallavier, France).

253 8 different column sets with a total of 27 analytical setups (gradient, modulation time) were
254 evaluated (cf. table 1).

255 4) Head-Space SolidPhase MicroExtraction (HS-SPME)

256 Given the volatility of most of the targeted compounds, injection was performed using HS-
257 SPME. Such a technique enabled to minimize the solvent peak compared to classical liquid
258 injection. This study was conducted with a focus on the separation and not on the limits of
259 detection. No quantitation was performed and the areas of the different peaks of interest
260 were not used. The SPME fiber used was a 75 µm DiVinylBenzene-Carboxen-
261 PolyDiMethylSiloxane (DVB-CAR-PDMS) from Supelco (Sigma–Aldrich, Saint Quentin Fallavier,
262 France). For HS-SPME procedures, standards were prepared by introducing 10 µL of the
263 mixture of 80 standards in the 20 mL vial. The fully automated HS-SPME procedure was as
264 follows. First, the vial was equilibrated at 70°C during 1 min, then the fiber was placed into the
265 head-space of the sample for the extraction, still maintained at 70°C for 20 min. At the end of
266 the extraction, the fiber was desorbed directly in the GC injector for 20 min at 250°C in split
267 mode (1:10).

268

269 IV) Results and discussion

270 In this paper, 27 different analytical setups were evaluated, an analytical setup being defined
271 by a column set, a gradient and a modulation time (cf. table 1). The optimization is mainly
272 about the column set, but the gradient and the modulation time had to be adapted to each
273 column set to get an enhanced separation, hence the relevance of the multicriteria strategy
274 implemented in this paper. A preliminary study was performed to define which ranges of

275 gradient and modulation time were relevant for each column set. The modulation time should
276 not exceed 10 seconds to avoid overloading the modulator jet, but it has to be long enough
277 to minimize wrapping around. In a first step, all analytical setups were tested with a gradient
278 of 3°C/min and a modulation time of 6 seconds. The eight column sets, the four different
279 gradients (2, 2.5, 3 or 4 °C/min) and the three different modulation times (6, 8 or 10 s) led to
280 a total of 96 different analytical setups, but a first selection was made as some analytical
281 setups were more promising than others. The fact that developed methods could be used for
282 routine analysis had also to be considered. Thus, data were processed with two different
283 objectives. First, criteria were chosen to get the best separation in a reduced run time. The
284 second objectives did not take the run time into account and gave more weight to the quality
285 of the separation.

286 1) Calculation of individual desirabilities

287 Prior to data processing, the bounds B_0 and B_1 were set (cf. table 2). The arithmetic mean of
288 NND provided information about peak spreading and extreme points, while the harmonic
289 mean provided a complementary information about peak clustering. In Nowik *et al.*, the
290 orthogonality of several data sets evaluated with the harmonic mean ranged from 0.017 to
291 0.054, a value of 0.047 being considered satisfactory. Hence the bounds B_1 and B_0 were set to
292 0.05 and 0.045 respectively. For the arithmetic mean, the values were in the interval [0.040 ;
293 0.084], hence our choice of the bounds B_1 and B_0 at 0.08 and 0.05 respectively. When the run
294 time was considered, analyses longer than 80 min (B_1) were put at disadvantage while
295 analyses which exceeded 150 were automatically discarded (B_0). As for the orthogonality
296 coefficient, the bounds B_0 and B_1 were set to 50 and 80% respectively. Concerning the
297 occupied space, B_0 and B_1 were set to 20 and 45% with raw data and to 30 and 55% with
298 normalized data.

299 Then, the individual desirabilities were calculated. Figure 2 shows the individual desirability
300 function of each criterion (blue line); the data of each analytical setup were plotted as red
301 crosses. Data spreading is different for each criterion showing the complementarity of the
302 selected criteria. Though data are clustered with the arithmetic mean, the use of the bounds
303 defined prior to the study (cf. table 2) was sufficient to discriminate between analytical setups
304 and make this criterion useful. With the Spearman correlation coefficient, the majority of data
305 receives complete fitness value, and only small numbers of analytical setups receive 0.5 or
306 some degrees between 0.5 and 1. These clusters show that a single criterion is not be sufficient
307 to rank the analytical setups properly. However, most criteria showed good spreading of data.

308 Finally, the calculation of the global desirability was performed with and without the time
309 constraint, leading to two different rankings.

310 2) Computation of the global desirability

311 2.1 Optimization for routine analyses with run time

312 Considering the run time, the calculation of occupied space with raw data was preferred to
313 the calculation with normalized data in order to account for possibly empty places at the
314 borders of the chromatograms. For instance, space can be lost at the end of the
315 chromatogram with too slow ramps of temperature. Moreover, the use of a column with non-

316 adapted polarities can lead to a loss of space covering which is not taken into account using
317 normalized data.

318 The quality of the separation was also evaluated with the number of detected compounds and
319 the number of coelutions.

320

321 2.2 Optimization regardless of run time

322 In this part, the objectives were to get the best separation regardless of run time (cf. table 2).

323 Thus, we chose to work with normalized data – as defined in *Statistical Analysis* - in order not
324 to consider empty space at the borders of chromatograms. Here, the quality of the separation
325 was favored over the run time. In this way, a satisfying peak scattering - even if on a limited
326 area of the chromatogram - was preferred. According to these choices, the number of
327 detected compounds and the number of coelutions were also used to evaluate the
328 performance of the separation.

329 3) Ranking of analytical setups

330 The exclusion of the run time criterion played a role but was shown not to be essential since
331 the same column set DB-1MS×DB-1701 – even with different analytical setups - appeared 3
332 times in the top 5, whatever the objective (cf. table 3). The column set DB-1MS×DB-1701
333 emerged as the most appropriate choice for the separation of the mixture of 80 compounds
334 (cf. chromatograms shown figures 3 and 4).

335 The column setup WAX×1701:3-6 ranked as fourth when run time was ignored. DB-WAX was
336 the most polar column at our disposal but was quickly discarded. Combined with a DB-1MS or
337 with a DB-5MS, it was expected to provide quite good results, because of the difference in
338 polarities. But the bleeding proved to be a true limitation for its use as a first dimension
339 column (cf. figure 5). When used as the second dimension column, an unacceptable echo
340 phenomenon occurred which had a strong impact on several analytical setups (1MS×WAX:3-
341 6, 5MS×WAX:3-6) evaluated in this study. The set DB-WAX×DB5 did not provide convincing
342 results but one of its analytical setups (WAX×5:3-6) was kept to see if it could give interesting
343 results anyway.

344 Prior to the experiments, only slight differences between the DB-1MS and the DB-5MS were
345 expected. But it is interesting to notice that most of the setups with a DB-5MS in first
346 dimension were discarded because of coelutions that were resolved using the DB-1MS. Thus,
347 only four setups with the DB-5MS×DB-1701 set were evaluated.

348 The use of an ionic liquid column (SLB-IL60) was expected to provide an enhanced separation
349 combined with a DB-1MS - or a DB-5MS - as its polarity is higher than DB-WAX's. However,
350 the preliminary tests showed it was not relevant for this study because of the dual-jet
351 cryomodulator. This column may be far too polar and the echo phenomenon was important
352 all over the chromatogram, thus invalidating the assumption of a phase modification due to
353 the cryomodulation. Moreover, even when the length was reduced from 1.5m to 1m, lots of
354 compounds kept on wrapping around even with the largest modulation time, leading to
355 broaden peaks. These two phenomena lead to eliminate this set because it was impossible to

356 identify compounds properly. With another modulation system – flow modulator or nitrogen
357 cryomodulator - the results might have been different. Another hypothesis is that the problem
358 is related to the analyte elution temperature from the first dimension: using a more polar
359 column might increase the analyte elution temperature, and, thereby, speed-up the elution in
360 the second dimension, thus avoiding wrap around. Using desirability is a true asset in this case
361 because the individual desirabilities of most criteria are acceptable for the 5MS×IL60:2-6
362 analytical setup. Considering that a lot of compounds were not found, the number of peaks
363 was low. But in this case, they were well scattered, leading to good results – according to the
364 desirability bounds - with the arithmetic mean, the harmonic mean, the correlation
365 coefficients and the orthogonality coefficient. Only the calculation of the surface coverage is
366 of particular interest here because the number of bins was chosen according to the number
367 of expected compounds. It gave interesting information about clustering, peak spreading and
368 took the number of peaks into account.

369 In the end, the same column set DB-1MS×DB-1701 with a gradient of 2.5°C/min - 2°C/min if
370 the run time is not considered - and a modulation time of 8 seconds appeared to be the best
371 analytical setup for the separation of the synthetic mixture of 80 compounds representative
372 of human hand odor. Moreover, this method was successfully applied to real samples and
373 provided good separations meeting our expectations, results which are the topic of a future
374 paper focusing on the sampling methods.

375 V) Conclusion

376 The use of comprehensive two-dimensional gas chromatography coupled with mass
377 spectrometry would be a true asset for the analysis of human odor. The evaluation of
378 multidimensional separation is not an easy task and various tools are available to assess the
379 orthogonality of the separation and choose a proper column set. A desirability analysis
380 allowed to consider more than one criterion and to rank the different column sets according
381 to predefined objectives. In this paper, 27 different analytical setups were evaluated using
382 nine criteria including six complementary orthogonality criteria: Spearman' correlation
383 coefficient, the harmonic and arithmetic means described by Nowik et al., the coefficient of
384 orthogonality described by Camenzuli et al. and the coverage of the separation space using
385 raw and normalized data. The use of desirability functions enabled to consider additional
386 simple but meaningful criteria such as the run time, the number of coelutions and the number
387 of detected compounds. Two desirability calculations were performed, one with an objective
388 of routine analysis and one without. Whatever the objectives, the same column set DB-
389 1MS×DB-1701 was found to be the most appropriate for the analysis of human hand odor.
390 This approach for the evaluation of orthogonality can be easily implemented with other
391 criteria (convex hull, synentropy, etc.) according to the operator's choice, i.e. considering the
392 targeted compounds and the application. The methods developed in this study are a solid
393 background for further experimentation and sampling methods development. They will
394 hopefully be used routinely with a large number of real samples for the analysis of human
395 hand odor to support the information provided by dogs in courts of justice.

396

References

- 398 [1] A.M. Curran, S.I. Rabin, K.G. Furton, Analysis of the uniqueness and persistence of
399 human scent, *Forensic Sci. Commun. Res. an Technol.* 7 (2005) 1–9.
- 400 [2] V. Cuzuel, G. Cognon, I. Rivals, C. Sauleau, F. Heulard, D. Thiébaud, J. Vial, Origin,
401 analytical characterization and use of human odor in forensics, *J. Forensic Sci.* 62 (2017)
402 330–350. doi:10.1111/1556-4029.13394.
- 403 [3] B. de Lacy Costello, A. Amann, H. Al-Kateb, C. Flynn, W. Filipiak, T. Khalid, D. Osborne,
404 N.M. Ratcliffe, A review of the volatiles from the healthy human body., *J. Breath Res.* 8
405 (2014). doi:10.1088/1752-7155/8/1/014001.
- 406 [4] J.S. Brown, P.A. Prada, A.M. Curran, K.G. Furton, Applicability of emanating volatile
407 organic compounds from various forensic specimens for individual differentiation.,
408 *Forensic Sci. Int.* 226 (2013) 173–182. doi:10.1016/j.forsciint.2013.01.008.
- 409 [5] Y. Hasegawa, M. Yabuki, M. Matsukane, Identification of new odoriferous compounds
410 in human axillary sweat., *Chem. Biodivers.* 1 (2004) 2042–50.
411 doi:10.1002/cbdv.200490157.
- 412 [6] S.K. Pandey, K.-H. Kim, Human body-odor components and their determination, *TrAC*
413 *Trends Anal. Chem.* 30 (2011) 784–796. doi:10.1016/j.trac.2010.12.005.
- 414 [7] M.R. Schure, J.M. Davis, Orthogonal separations: Comparison of orthogonality metrics
415 by statistical analysis, *J. Chromatogr. A.* 1414 (2015) 60–76.
416 doi:10.1016/j.chroma.2015.08.029.
- 417 [8] R. Al Bakain, I. Rivals, P. Sassi, D. Thiébaud, M.-C. Hennion, G. Euvrard, J. Vial,
418 Comparison of different statistical approaches to evaluate the orthogonality of
419 chromatographic separations: application to reverse phase systems., *J. Chromatogr. A.*
420 1218 (2011) 2963–75. doi:10.1016/j.chroma.2011.03.031.
- 421 [9] E. Van Gysegem, I. Crosiers, S. Gourvéne, D.L. Massart, Y. Vander Heyden,
422 Determining orthogonal and similar chromatographic systems from the injection of
423 mixtures in liquid chromatography-diode array detection and the interpretation of
424 correlation coefficients color maps, *J. Chromatogr. A.* 1026 (2004) 117–128.
425 doi:10.1016/j.chroma.2003.10.116.
- 426 [10] T.J. Whelan, M.J. Gray, P.J. Slonecker, R.A. Shalliker, M.A. Wilson, Study of the
427 selectivity of reversed-phase columns for the separation of polycarboxylic acids and
428 polyphenol compounds, *J. Chromatogr. A.* 1097 (2005) 148–156.
429 doi:10.1016/j.chroma.2005.08.026.
- 430 [11] S. Zhu, Effect of column combinations on two-dimensional separation in
431 comprehensive two-dimensional gas chromatography: Estimation of orthogonality and
432 exploring of mechanism, *J. Chromatogr. A.* 1216 (2009) 3312–3317.
433 doi:10.1016/j.chroma.2009.01.107.
- 434 [12] M. Gilar, J. Fridrich, M.R. Schure, A. Jaworski, Comparison of orthogonality estimation
435 methods for the two-dimensional separations of peptides, *Anal. Chem.* 84 (2012) 8722–
436 8732. doi:10.1021/ac3020214.
- 437 [13] P.J. Slonecker, X. Li, T.H. Ridgway, J.G. Dorsey, Informational orthogonality of two-

- 438 dimensional chromatographic separations, *Anal. Chem.* 68 (1996) 682–689.
439 doi:10.1021/ac950852v.
- 440 [14] P.J. Clark, F.C. Evans, Distance to nearest neighbor as a measure of spatial relationships
441 in populations, *Ecology*. 35 (1954) 445–453.
- 442 [15] D. Brown, P. Rothery, Randomness and local regularity of points in a plane, *Biometrika*.
443 65 (1978) 115–122. doi:10.1093/biomet/65.1.115.
- 444 [16] W. Nowik, S. Héron, M. Bonose, M. Nowik, A. Tchaplá, Assessment of two-dimensional
445 separative systems using nearest-neighbor distances approach. Part 1: Orthogonality
446 aspects, *Anal. Chem.* 85 (2013) 9449–9458. doi:10.1021/ac4012705.
- 447 [17] W. Nowik, M. Bonose, S. Héron, M. Nowik, A. Tchaplá, Assessment of two-dimensional
448 separative systems using the nearest neighbor distances approach. Part 2: Separation
449 quality aspects, *Anal. Chem.* 85 (2013) 9459–9468. doi:10.1021/ac4012717.
- 450 [18] M.R. Schure, J.M. Davis, The statistical overlap theory of chromatography using power
451 law (fractal) statistics, *J. Chromatogr. A.* 1218 (2011) 9297–9306.
452 doi:10.1016/j.chroma.2011.10.013.
- 453 [19] M. Gilar, P. Olivova, A.E. Daly, J.C. Gebler, Orthogonality of separation in two-
454 dimensional liquid chromatography, *Anal. Chem.* 77 (2005) 6426–6434.
455 doi:10.1021/ac050923i.
- 456 [20] J. Leonhardt, T. Teutenberg, G. Buschmann, O. Gassner, T.C. Schmidt, A new method
457 for the determination of peak distribution across a two-dimensional separation space
458 for the identification of optimal column combinations, *Anal. Bioanal. Chem.* 408 (2016)
459 8079–8088. doi:10.1007/s00216-016-9911-3.
- 460 [21] D. Ryan, P. Morrison, P. Marriott, Orthogonality considerations in comprehensive two-
461 dimensional gas chromatography, *J. Chromatogr. A.* 1071 (2005) 47–53.
462 doi:10.1016/j.chroma.2004.09.020.
- 463 [22] G. Semard, V. Peulon-Agasse, A. Bruchet, J.P. Bouillon, P. Cardinaël, Convex hull: A new
464 method to determine the separation space used and to optimize operating conditions
465 for comprehensive two-dimensional gas chromatography, *J. Chromatogr. A.* 1217
466 (2010) 5449–5454. doi:10.1016/j.chroma.2010.06.048.
- 467 [23] S.C. Rutan, J.M. Davis, P.W. Carr, Fractional coverage metrics based on ecological home
468 range for calculation of the effective peak capacity in comprehensive two-dimensional
469 separations, *J. Chromatogr. A.* 1255 (2012) 267–276.
470 doi:10.1016/j.chroma.2011.12.061.
- 471 [24] Z. Liu, D. Patterson Jr, M.L. Lee, Geometric Approach to Factor Analysis for the
472 Estimation of Orthogonality and Practical Peak Capacity in Comprehensive
473 TwomDimensional Separations, *Anal. Chem.* 67 (1995) 3840–3845.
474 doi:10.1021/ac00117a004.
- 475 [25] Z. Da Zeng, H.M. Hugel, P.J. Marriott, A modeling approach for orthogonality of
476 comprehensive two-dimensional separations, *Anal. Chem.* 85 (2013) 6356–6363.
477 doi:10.1021/ac400736v.
- 478 [26] A. Mani-Varnosfaderani, M. Ghaemmaghami, Assessment of the orthogonality in two-
479 dimensional separation systems using criteria defined by the maximal information

- 480 coefficient, *J. Chromatogr. A.* 1415 (2015) 108–114. doi:10.1016/j.chroma.2015.08.049.
- 481 [27] J. Jacova, A. Gardlo, D. Friedecky, K. Hron, T. Adam, J.-M.D. Dimandja, Evaluation of
482 orthogonality assessment tools in GCxGC - 13th GCxGC Symposium (Riva del Garda),
483 (2016).
- 484 [28] C. Cordero, P. Rubiolo, B. Sgorbini, M. Galli, C. Bicchi, Comprehensive two-dimensional
485 gas chromatography in the analysis of volatile samples of natural origin: A
486 multidisciplinary approach to evaluate the influence of second dimension column
487 coated with mixed stationary phases on system orthogonality, *J. Chromatogr. A.* 1132
488 (2006) 268–279. doi:10.1016/j.chroma.2006.07.067.
- 489 [29] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-
490 Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Elsevier Ltd, 1998.
- 491 [30] J. Vial, M. Cohen, P. Sassi, D. Thiébaud, Pharmaceutical quality of docetaxel generics
492 versus originator drug product : a comparative analysis, *Curr. Med. Reserach Opin.* 24
493 (2008) 2019–2033. doi:10.1185/03007990802207874.
- 494 [31] G. Derringer, R. Suich, Simultaneous optimization of several response variables., *J. Qual.*
495 *Technol.* 12 (1980) 214–219.
- 496 [32] M. Camenzuli, P.J. Schoenmakers, A new measure of orthogonality for multi-
497 dimensional chromatography, *Anal. Chim. Acta.* 838 (2014) 93–101.
498 doi:10.1016/j.aca.2014.05.048.
- 499 [33] S. Varala, B. Dharanija, B. Satyavathi, V. V. Basava Rao, R. Parthasarathy, New
500 biosorbent based on deoiled karanja seed cake in biosorption studies of Zr(IV):
501 Optimization using Box-Behnken method in response surface methodology with
502 desirability approach, *Chem. Eng. J.* 302 (2016) 786–800.
503 doi:10.1016/j.cej.2016.05.088.
- 504 [34] A.L. Bukzem, R. Signini, D.M. dos Santos, L.M. Lião, D.P.R. Ascheri, Optimization of
505 carboxymethyl chitosan synthesis using response surface methodology and desirability
506 function, *Int. J. Biol. Macromol.* 85 (2016) 615–624.
507 doi:10.1016/j.ijbiomac.2016.01.017.

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510 Figures and tables captions, in order of appearance

511

512 Figure 1: Example of a desirability plot

513

514 Table 1: Analytical setups

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516 Table 2: Bounds used for individual desirability calculations

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518 Figure 2: Individual desirabilities plot

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520 Table 3: Ranking of analytical setups

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522 Figure 3: Comprehensive GC×GC-MS chromatogram –mixture of 80 standards – DB-1MS×DB-
523 1701 – modulation time: 8s – gradient: 2.5°C/min (detailed conditions in the experimental
524 part)

525

526 Figure 4: Comprehensive GC×GC-MS chromatogram –mixture of 80 standards – DB-1MS×DB-
527 1701 – modulation time: 8s – gradient: 2°C/min (detailed conditions in the experimental
528 part)

529

530 Figure 5: Comprehensive GC×GC-MS chromatogram –mixture of 80 standards – DB-
531 WAX×DB-1701 – modulation time: 8s – gradient: 3°C/min (detailed conditions in the
532 experimental part)

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