

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Current Developments in Analyzing Food Volatiles by Multidimensional Gas Chromatographic Techniques

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1662591> since 2018-03-16T14:45:19Z

Published version:

DOI:10.1021/acs.jafc.6b04997

Terms of use:

Open Access

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

(Article begins on next page)

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

[Chiara Cordero, Hans-Georg Schmarr, Stephen E.Reichenbach and Carlo Bicchi; **Current**

Developments in Analyzing Food Volatiles by Multidimensional Gas

Chromatographic Techniques;]

The publisher's version is available at:

[10.1021/acs.jafc.6b04997]

When citing, please refer to the published version.

Link to this full text:

[hdl:2318/1662591]

This full text was downloaded from iris-Aperto: <https://iris.unito.it/>

Current Developments in Analyzing Food Volatiles by Multidimensional Gas

Chromatographic Techniques

Chiara Cordero*¹, Hans-Georg Schmarr*^{2,3}, Stephen E.Reichenbach⁴ and Carlo Bicchi¹

Authors' affiliation:

¹ Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Turin, Italy

² Dienstleistungszentrum Ländlicher Raum (DLR) – Rheinpfalz; Institut für Weinbau und Oenologie;

Breitenweg 71, D-67435 Neustadt an der Weinstraße

³ University Duisburg-Essen; Faculty of Chemistry; Instrumental Analytical Chemistry,

Universitätsstraße 5, 45141 Essen, Germany

⁴ University of Nebraska – Lincoln, Lincoln NE 68588-0115, USA

* Address for correspondence:

Prof. Dr. Chiara Cordero - Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino,

Via Pietro Giuria 9, I-10125 Torino, Italy – e-mail: chiara.cordero@unito.it ; phone: +39 011

6707662; fax: +39 011 2367662

PD Dr. Hans-Georg Schmarr - Dienstleistungszentrum Ländlicher Raum (DLR) – Rheinpfalz; Institut

für Weinbau und Oenologie; Breitenweg 71 D-67435 Neustadt an der Weinstraße, Germany – e-

mail: hans-georg.schmarr@dlr.rlp.de; phone: +49-(0)-6321-671-289; fax: +49-(0)-6321-671-375

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional
Repository

1 **Abstract**

2 This paper presents current developments and future perspectives on the spreading of advanced
3 analytical tasks in the field of food volatile analysis. The topics outlined comprise: (a) recent
4 advances on miniaturized sampling techniques; (b) the potential and challenges of
5 multidimensional gas chromatography coupled with mass spectrometric detection for volatile
6 identification and quantitation in samples with complex matrices; (c) the potential of
7 comprehensive two-dimensional gas chromatography in fingerprinting studies, in particular for
8 classifying complex samples in routine analysis; and (d) the key role of dedicated software tools for
9 data elaboration with comprehensive two-dimensional separations.

10

11

12 **Key-words:**

13 Analysis of food volatiles; sample preparation; multidimensional gas chromatography;
14 comprehensive two-dimensional gas chromatography; fingerprinting

15

16 Introduction

17 Although understanding of food aroma comprises the knowledge about its volatile and
18 non-volatile fractions, in this work, we focus our discussion on the analysis of volatile compounds.
19 Concepts for the analyses and understanding of the contributions of non-volatiles to food aroma
20 can be found elsewhere.¹

21 Basically, food volatile analyses can be differentiated into targeted analysis of key odorants
22 and profiling or fingerprinting analyses, targeting at best the sum of volatile compounds amenable
23 to the chosen analytical technique. Fingerprinting analyses often involve *-omics* strategies tackling,
24 for example, food sensory quality characterization (*sensomics*, *flavor metabolomics* and
25 *flavoromics*).^{2,3} A focus usually is on sensory-active compounds to correlate stimuli of multimodal
26 perceptions of food (i.e., aroma, taste, texture, etc.) with specific and peculiar chemical signatures,
27 i.e., the chemical pattern associated with the perceived property. In this perspective,
28 comprehensive investigations are required to combine chemical information on sample
29 components (analyte identity and concentration in the matrix) with their sensory quality.

30 Well established investigation protocols, such as the molecular sensory science approach,⁴
31 focus on isolation, identification, and quantitation of key-aroma compounds by combining
32 extraction (e.g., liquid-liquid extraction (LLE), solvent assisted flavor evaporation (SAFE),
33 simultaneous distillation extraction (SDE), solid phase extraction (SPE), or supercritical fluid
34 extraction (SFE)) and analysis by gas chromatographic separation and mass spectrometric
35 detection (GC-MS), odorant detection by GC-Olfactometry (GC-O), and structure elucidation. An
36 important part of the characterization of key-aroma compounds is their accurate quantitative
37 measurement, together with the knowledge of their sensory threshold in the respective food.
38 Each of these discrete and often manifold analytical operations can be difficult, e.g., due to the
39 very low concentration of potent odorants in complex matrices, but are fundamental to detailing

40 flavor-chemical signatures. The option and possibility of extending such detailed protocols to high-
41 throughput screening and/or fingerprinting would be very useful and attractive.⁵

42 Based on their experience, the authors here present their viewpoint and outline
43 perspectives on the future of advanced approaches in the field of volatile aroma analysis. The
44 following steps within the analytical work-flow are addressed: (a) recent advances on miniaturized
45 sampling techniques; (b) the potential and challenge of multidimensional (MD) GC-MS for volatile
46 identification and quantitation in samples with complex matrices; (c) the potential of
47 fingerprinting studies with comprehensive two-dimensional gas chromatography (GC×GC), in
48 particular for routine analyses to classify complex samples; and (d) the key role of dedicated
49 software tools for data elaboration with comprehensive two-dimensional separations.

50

51 **Recent advances in sample preparation**

52 Sample preparation usually is the beginning of an analytical workflow and thus may be
53 considered as the first dimension of the total analysis system. In multi-dimensional separations,
54 with the first separation usually assigned to the first dimension, so sample preparation could be
55 designated the zeroth dimension, as it presents an initial discrimination of the analytes in function
56 of one specific characteristic (polarity, volatility, etc.). Sample preparation should match with the
57 sample's chemical dimensions⁶ to deliver a meaningful picture of its constituents, allowing access
58 to the multiple levels of information.

59 The so-called "aroma-active compounds" are part of the volatile and semi-volatile fraction
60 of a sample and their distribution is highly informative for its sensory quality. Extraction and
61 sampling conditions should be carefully considered to meet fundamental requirements. A
62 sampling system capable of mapping odor-active compounds should have: (a) an appropriate, and
63 possibly tunable, extraction selectivity; (b) high extraction efficiency/capability to capture trace

64 and ultra-trace analytes (with high odor impact); (c) extraction mechanisms based on mild
65 interactions (sorption/partition is preferable to adsorption) to limit artifact formation (e.g., as
66 often seen in thermo-desorption at higher temperatures); and (d) a good integration into the
67 instrumental analytical system, including the software-assisted automation of all operation
68 steps.^{5,7,8} The choice and optimization of sample preparation depends on the fundamental
69 question(s) driving the analysis. In some cases, the goal is a more or less holistic understanding of
70 the sample's nature; then, a comprehensive extraction comprising as many compounds as possible
71 is required. In other cases, the goal may be a target analysis, e.g., the common problem in off-
72 flavor analysis. Then, a more specific extraction is preferred to reduce the concentration of
73 unwanted matrix compounds and possibly enrich the target analyte(s). Affinity chromatography,
74 e.g., for the selective enrichment of thiols (sulfanyls), is an interesting example in this respect.⁹

75 Headspace sampling approaches, particularly those with a concentration step (high
76 concentration capacity headspace techniques, HCC-HS), often are preferred for satisfactory
77 throughput in volatile aroma analysis. Headspace solid phase microextraction (HS-SPME) is the
78 most popular HCC techniques and its hyphenation with gas chromatography is extensively
79 documented in literature.^{7,8,10-12} A recent advancement of this technique in flavor analysis is the
80 so-called SPME Arrow,^{13,14} which has increased sorption phase volumes (from 0.5-1 μ L of standard
81 SPME fibers to about 15 μ L of SPME Arrows) and overall mechanical stability. A schematic of the
82 commercial device is illustrated in Figure 1. The higher amount of sorption phase(s) provide(s) an
83 improved sensitivity and polymer chemistry (polydimethylsiloxane/carboxen 1000 - PDMS/CAR
84 1000; PDMS/Carbon WR -PDMS/CAR WR; polyacrylate -PA; polydimethylsiloxane - PDMS) thereby
85 enabling better tuning of extraction selectivity for analytes of interest.

86 Preliminary studies with the SPME Arrow for profiling volatile amines in water samples and
87 in the atmosphere¹⁵ achieved better sensitivity and robustness compared to conventional SPME.

88 Limited effects of competitive adsorption with complex samples were observed. This aspect is of
89 particular interest for profiling volatiles because thermally transformed food (coffee, cocoa,
90 roasted fruits, etc.) exhibit very complex patterns that also exert strong matrix effects on
91 headspace composition.

92 Another interesting study by Kremser et al.¹⁴ systematically compared the effectiveness of
93 the SPME Arrow with established static and dynamic headspace techniques. Results on a model
94 mixture of volatiles covering a wide range of polarity and volatility (from C2 to C10), confirmed its
95 efficiency in recovering a wide range of volatiles from the sample HS and showed relative
96 recoveries comparable or even better than SPME.

97 Conventional SPME, combined with multidimensional GC separations is very common¹⁶⁻²²
98 although its extraction capability is sometimes limiting for ultra-trace odorant analysis. In an
99 interesting experiment to enhance method sensitivity for GC-O in wine aroma assessment, Chin et
100 al.²³ proposed cumulative multiple HS-SPME samplings with two different fiber coatings, followed
101 by successive GC injections delayed over time. Desorbed volatiles were collected at the first
102 section of the separation column using cryo-trapping (CT) to guarantee short initial band widths
103 for analysis. This experimental design aimed at matching sensitivity requirements for GC-O
104 screening, although it presented challenging aspects: the difficulty of automation and of
105 performing replicate assays or dilution experiments with GC-O. With the complex wine matrix and
106 a one-dimensional separation, the authors also addressed the necessity for an improved
107 separation strategy to better separate individual compounds. New developments in this field of
108 aroma analysis are discussed in more detail in the next paragraph.

109 Higher HS extraction efficiency also can be achieved by dynamic headspace (D-HS) or by
110 adopting sampling approaches with a higher amount of extraction phase. In a study aimed at
111 characterizing the volatile fraction from dried milk samples, Cordero et al.²⁴ reported a systematic

112 investigation on different but complementary sampling techniques, based on either sorption or
113 adsorption, or on their combination. The approaches investigated show a high degree of
114 automation, and included SPME, stir bar sorptive extraction (SBSE) and headspace sorptive
115 extraction (HSSE) with PDMS and dual phase SBSE, and D-HS with silicone sorbents or polar
116 adsorbents (e.g., Tenax TA™). Information for analytes, including key-odorants and off-odors,
117 extracted by headspace and in-solution sampling were compared to evaluate whether a given
118 orthogonal approach was advantageous to describe samples' sensory properties. Within
119 headspace approaches, HCC techniques with higher amounts of polymeric accumulation phase
120 (i.e., HSSE and D-HS) gave better results in terms of concentration capacity. Single-polymer SPME
121 fibers, including polar and selective polymers (i.e., polyacrylate-PA and polyethylene glycol - PEG),
122 were less effective than multi-polymer devices (e.g., DVB/CAR/PDMS SPME). With in-solution
123 sampling, the concentration capacity of SBSE was superior for both sampling systems (100% PDMS
124 and dual-phase PDMS Carboxen 100), achieving concentration factors of 6 to 7 times compared
125 to HCC-HS techniques. This aspect is crucial for an integrated analytical platform for sensomics,
126 where GC-O should be carried out contemporarily to the identification and quantitation of odor-
127 active compounds. However, in-solution sampling by SBSE or SPME has to be carefully evaluated
128 for aroma analysis being prone to artifacts formation, during thermal-desorption of analytes, due
129 to the presence of aroma precursors in the sample, and to possible recovery discrimination due to
130 the different analyte/accumulating phase interaction.

131

132 **Exploiting multidimensional separation in targeted aroma analysis**

133 Multidimensional gas chromatography (MDGC) plays a crucial role in flavor research either
134 in the conventional heart-cut (H/C MDGC) or in comprehensive (GC×GC) mode.²⁵ H/C MDGC has
135 long been used, although its widespread application still remains unfulfilled. Behind the

136 development of MDGC, in the early days of capillary GC, there was the need for well separated
137 chromatographic peaks for accurate quantitative analyses or for the characterization of complex
138 fractions, including enantiomeric recognition for authentication purposes. Despite instrumental
139 advancements and full automation of heart-cut (H/C) procedures, H/C MDGC is still considered a
140 niche technique for applications in which 1D-GC does not offer sufficient separation power or
141 when the hyphenation with MS (including tandem MS) does not provide the required level of
142 information (e.g. enantiomer recognition).

143 In a study aimed at investigating the enantiomeric distribution of a potent aroma
144 compound, 3-sec-butyl-2-methoxypyrazine (SBMP), in a number of vegetal and fruit species, H/C
145 MDGC was used for chiral recognition with heptakis-(2,3-di-*O*-methyl-6-*O*-tert-butyl)dimethylsilyl)-
146 β -cyclodextrin as chiral selector in the second dimension (²D). Sensory evaluation of the individual
147 (*R*)- and (*S*)-SBMP did not show differences in their odor quality, but their odor thresholds differ by
148 an order of magnitude, 0.01 and 0.1 ppb in water for (*R*)- and (*S*)-SBMP, respectively.

149 Legrum et al.²⁶ described the chiral recognition of SBMP with different GC-MS systems.
150 Trace-level analysis (ng/L or ng/kg) of SBMP in lady beetles and *Vitis vinifera* species was
151 successfully performed with a single-oven H/C MDGC system with a cryo modulator (as cold trap)
152 for trapping analytes transferred from the achiral first-dimension (¹D) column to the chiral second-
153 dimension (²D) column before starting the ²D enantioseparation with an independent temperature
154 ramp. Highly selective detection was achieved with a triple quadrupole MS (QqQ-MS) in MS/MS
155 mode by selecting suitable mass transitions. Figure 2 illustrates the system proposed by Legrum et
156 al.²⁶ for this study. Experimental results confirmed discrimination of the (*R*)- and (*S*)- enantiomers
157 of SBMP and revealed that only (*S*)-SBMP was detected. This result supports the hypothesis of
158 natural amino acids serving as starting material for the biosynthesis of alkyl-methoxypyrazines. For
159 higher concentration levels ($\mu\text{g}/\text{Kg}$) such as those found in matrices from peppers, carrots, sugar

160 peas, and potatoes, a classical H/C MDGC system with two independent GC ovens and selected ion
161 monitoring (SIM) with a single quadrupole MS (qMS) usually was sufficient to achieve reliable
162 quantitative data. However, the authors mention co-elution problems within some matrices (e.g.,
163 parsnips), that would demand either a more selective detection mode (MS/MS) or a better
164 separation (or both). At trace-level alkyl-methoxypyrazine analysis, selective MS detection was
165 necessary for complex samples and/or for accurate quantitation below 1 µg/Kg. In these cases,
166 selective reaction monitoring (SRM) by QqQ-MS was succesful.^{26,27}

167 GC×GC can today be considered as the state-of-the-art technique in terms of separation
168 efficiency. Legrum et al. achieved the enantiomer separation of SBMP with a GC×GC system that
169 automatically trapped analytes eluting from the chiral ¹D column and re-injected them into the
170 achiral ²D column with a dual-jet cryo modulator and monitored characteristic fragment ions with
171 a time-of-flight mass spectrometer (ToF-MS). They also discussed the influence of the modulation
172 period that must be short for such enantioseparations with limited chiral resolution and the
173 alternative of using a GC×enantioGC system. Besides the separation efficiency of GC×GC in
174 general, trace-level analysis can further benefit from spectral deconvolution algorithms more
175 effective with ToF-MS data.²⁸ In general, data quality obtained in such trace analysis benefits from
176 additional analytical refinements and data obtained with less analytical effort in some cases may
177 be wrong with critical matrices.^{29,30}

178 Several challenging aspects are noteworthy in order to optimize H/C MDGC analyses. The
179 most important aspect of H/C MDGC is probably to increase the separation selectivity for target
180 compounds within a complex matrix. Defining the H/C temporal window then should minimize the
181 risk for co-transferring eventually disturbing matrix compounds. A careful definition of such H/C
182 temporal windows for a quantitative transfer of both analyte and isotopologue is mandatory in
183 particularly when quantification via a stable isotope dilution assay (SIDA) is intended, and ¹D

184 separation conditions as well as isotopic labeling should be optimized. The reason lies in the
185 chromatographic separation of isotopologues that often shows an *isotope* effect,³¹ thus a
186 retention shift along the ¹D separation that demands an elongation of the temporal H/C window
187 to avoid losses of analyte and/or the isotopic internal standard. Then, a partial transfer of either
188 ones results in inaccurate quantifications. This should be avoided because an increase of the
189 temporal transfer windows enhances the risk for transferring disturbing matrix compounds.
190 Selecting an appropriate isotopic labeling strategy and optimized conditions for the
191 chromatographic separation, notably choosing an appropriate polarity for the separation column
192 stationary phase, then affords a complete or almost complete co-elution situation on the ¹D. Such
193 optimization thus allows for a minimized H/C temporal window and a reliable SIDA-based
194 quantification.³²

195 In the field of food adulteration, Langen et al.³³ proposed an interesting application of H/C
196 MDGC for quantitative determinations of α - and β -ionone and β -damascenone and enantiomeric
197 separation of α -ionone in wine samples. These potent odorants, deriving from carotenoids
198 cleavage, are reminiscent of violet, raspberry, and floral flavor attributes, and generally are
199 present as key-odorants in raspberries, tea, and tobacco. In wine, when present, their
200 concentrations vary between a few micrograms and 60 $\mu\text{g/L}$.³³ Their impact on wine aroma is
201 favored by an interesting synergism with other odorants (e.g., ethyl cinnamate and hexanoate)
202 also inducing a masking effect against the herbaceous, bell-pepper like aroma of isobutyl
203 methoxypyrazine.³⁴ The control authorities consider the assessment of the enantiomeric
204 distribution of chiral aroma compounds as a point of interest to reveal adulterations by artificial
205 aroma. In order to enhance the distinctive raspberry note often found in rosé wines, α -ionone
206 might be added with a fraudulent purpose. In natural raspberry, α -ionone occurs primarily as the
207 (*R*)-enantiomer. This also is found in wines made from *Vitis vinifera* varieties, together with low

208 amounts of β -ionone and β -damascenone. In this respect, concentration levels and enantiomeric
209 distribution of chiral compounds are excellent markers for authenticity studies. In order to achieve
210 low detection limits, Langen et al.³³ successfully applied an optimized H/C MDGC with an
211 enantioselective GC column in the ²D and a highly selective QqQ-MS detector (Limit of
212 Quantitation (LOQ) were 0.007 μ g/L for (*S*)- and (*R*)- α -ionone, 0.016 μ g/L for β -ionone and 0.026
213 μ g/L for β -damascenone). Accurate quantitation was achieved by SIDA in a set of 95 red, 75 rosé,
214 and 64 white wines and revealed the presence of most of these key odorants in the range
215 between method LOQ and 10 μ g/L. The (*R*)- α -ionone clearly dominates in authentic wines; the *R/S*
216 ratio could be adopted as a good indicator of wine (aroma) authenticity.

217 During their method development, Langen et al.³³ showed that a careful selection of a
218 suitable MS/MS transition is also necessary for unambiguous identification of (*S*)- and (*R*)- α -ionone
219 – even after H/C MDGC as an efficient method for matrix reduction. The reliability of the
220 quantitation accuracy is affected by an interfering peak eluting between the two enantiomers on
221 the enantioselective (ES) ²D column (here heptakis-(2,3-di-*O*-methyl-6-*O*-tert-butyl-dimethylsilyl)- β -
222 cyclodextrin). Figure 3 shows the different ²D profiles corresponding to three specific transitions
223 (SRM) in the elution region of (*S*)- and (*R*)- α -ionone.

224 The authors discussed the critical selection of precursor ions for further fragmentation in
225 the context of the low molecular weight and often highly fragmented spectra of aroma
226 compounds with classical electron impact ionization (EI). They propose favoring the highest
227 possible mass for MS-MS aroma analyses, as otherwise ubiquitous small mass fragments may lead
228 to nonselective SRM transitions that could yield erroneous results. In volatile aroma analysis from
229 complex matrices, this should be considered and often calls for a selective sample preparation,
230 increased separation efficiency (e.g. by H/C MDGC), and a very selective detection. In a recent
231 work, the authors thus proposed for a relatively simple determination of 2-aminoacetophenon in

232 wine on $\mu\text{g/L}$ an application of H/C MDGC-MS-MS, as otherwise erroneous results may occur in
233 some wines when the separation efficiency or detection selectivity is lowered to a 1D approach.³⁵

234 The fundamental drawback of small fragment ions encountered with volatile aroma
235 compounds might be overcome by soft ionization techniques such as classical chemical ionization
236 (CI) or new developments, e.g., the "Cold-EI" technique that is based on a supersonic molecular
237 beam (SMB)³⁶ (Aviv Analytical, Tel Aviv, Israel) or the "Select-eV" ion source that uses a novel
238 electron lens for maintaining high ionization efficiency at low eV levels (Markes International,
239 Llantrisant, UK). However, at present, Select-eV is not yet offered with MS/MS detection. On the
240 other hand, Select-eV already can be used in a fast switching ionization mode, named tandem
241 ionization, which affords two sets of MS data to be acquired and further processed. Applications
242 for Select-eV have been described in the petrochemical field³⁷ but also in aroma analysis³⁸. An
243 example of mass spectral signatures with enhanced molecular ions for ethylphenols in wine is
244 provided as supplementary material in Supplementary Figure 1 (SF1).

245 High resolution MS (HR-MS) represents an ultimate option for selective detection leading
246 to unambiguous structure elucidation or confirmation. Marketed solutions for GC hyphenation are
247 available from Agilent, Waters, LECO and ThermoFisher). A clear example of the potentials is given
248 by recent experiments on aroma active compounds by GC-HR-MS and Orbitrap. The Orbitrap
249 technology had earlier been established for LC-HR-MS and matured over the past years. Technical
250 specifications for the GC-Orbitrap claim a mass resolution of 12.500 to 100.000 (18 Hz to 3 Hz) at
251 $m/z = 272$ (ThermoFisher Scientific, Dreieich, Germany). These systems are especially suitable for
252 structure elucidation, as shown for β -damascenone and α -ionone with the mass fragment of $m/z =$
253 121 common to both. The exact mass reveals that $m/z = 121.06480$ in the case of α -ionone
254 contains an oxygen atom that is compatible with the structure shown in the supplementary
255 material (Supplementary Figure SF2). At present, no application in the aroma field has been

256 published, but one can expect the beneficial use of HR-MS in the near future – although, at the
257 high costs for such detection systems.

258

259 **Exploiting multidimensional separation in non-targeted aroma analysis**

260 Highly specific and targeted H/C MDGC methodologies only partially answer the analytical
261 needs of detailed analysis of volatiles fractions in aroma chemistry. A number of studies based on
262 multidimensional separations followed by non-targeted data interpretation methodologies have
263 demonstrated the informative potentials of such detailed chemical patterns.⁵ Since its
264 introduction by Liu and Phillips in 1991,³⁹ it was immediately evident that GC×GC would provide
265 substantial advantages for detailed characterizations of complex mixtures such as some food-
266 derived volatile fractions, containing odor-active compounds responsible for sensory attributes.
267 These advantages derive from the adoption of (almost) orthogonal separation mechanisms in the
268 two dimensions of the separation system. Resulting patterns show ordered structures for
269 chemically related analytes and are helpful for group-type analysis or for identification purposes.

270 Figure 4 shows the 2D pattern of volatiles from an extra-virgin olive (EVO) oil sample.
271 Figure 4A reports the full pattern of the volatile fraction separated on a GC×GC system with a poly
272 ethylene glycol ¹D column (PEG) and a medium polarity ²D column (OV1701). Figure 4B localizes
273 the group of target analytes (identified through their EI-MS fragmentation pattern and linear
274 retention index I^T_s). Figure 4C illustrates the ordered spatial distribution of homologues series and
275 groups of analytes (esters, saturated and unsaturated aldehydes, hydrocarbons, and alcohols).

276 Adahchour et al.^{40,41} were early investigators of the potential of GC×GC in the field of
277 aroma characterization. They investigated aroma extracts from milk-derived products (dairy and
278 non-dairy sour cream and dairy spread) obtained by SAFE and CF (cold finger) distillation. The
279 analytical platform was equipped with a thermal modulator (a longitudinally modulated cryogenic

280 system - LMCS). The authors convincingly showed the merits of the technique in providing
281 valuable information about volatiles distribution (profiling) at the same time informing on the
282 presence of key-flavor components in milk-derived samples. The enhanced overall
283 chromatographic resolution facilitated accurate quantitation of a selection of target compounds,
284 such as methional and sotolon, found in the milk-product extracts at mg/kg concentration. The
285 need for further improvements of the technique by designing alternative separation strategies
286 were the seeds of subsequent instrumental developments that made the technique so successful
287 in food investigations.^{16,42}

288 The so called "multi-multidimensional" platforms, introduced by Marriott and co-workers,
289 ⁴³⁻⁴⁶ effectively combine most of well established GC related techniques adopted in flavor analysis.
290 An example of a system implementing H/C MDGC, GC×GC and GC-O is reported in the
291 supplementary material as Supplementary Figure SF3.

292 Maikhunthod et al.⁴⁶ presented a platform implementing a switchable device between
293 comprehensive two-dimensional gas chromatography and targeted multidimensional gas
294 chromatography system (i.e., switchable GC×GC/targeted MDGC). The system enabled
295 independent analyses by 1D-GC, GC×GC, and targeted MDGC with the possibility of switching from
296 GC×GC to targeted MDGC at any times throughout a single analysis. A Deans switch microfluidic
297 transfer module and a cryotrapping device (CT) are core components enabling classical H/C
298 operations, GC×GC, or targeted MDGC. System operational performances were evaluated by
299 analyzing volatiles of interest in the flavor and fragrance field and on medium complexity essential
300 oils. Experiments were mainly focused on obtaining better resolved peaks by a targeted separation
301 on a longer 2D column by diverging specific regions of a GC×GC separation in which co-elutions
302 occurred, thus allowing reliable identification and quantitation of target analytes.

303 The potentials of hyphenated and multi-multidimensional systems to study aroma-impact

304 compounds were extensively illustrated by Cordero et al.⁵ in an article reviewing the existing
305 literature with an eye to the future. The authors touted the still unexplored potential of
306 comprehensive GC in food fingerprinting investigations. The dense and multidimensional data sets
307 produced by each single analytical run necessitate suitable data mining approaches that expand
308 the simple targeted investigation methodology. Clear benefits of combined targeted and
309 untargeted fingerprinting are obtained in the area of complex samples classification and
310 discrimination. Some examples will be dealt with in the following discussion on data elaboration.

311 Another area of active research in the field of GC×GC is to develop effective modulation
312 devices that are cheaper and consumable-free and so suitable for adoption of GC×GC in routine
313 applications and quality control (QC) procedures. The characterization of key odorants requires
314 devices for efficient trapping and release of (highly) volatile components, most of them
315 responsible for distinct odor notes. Dual-stage thermal modulators (TM) with cooling media (CO₂,
316 liquid N₂ or closed cycle refrigerator/heat exchangers) prevail because of their flexibility and ability
317 to produce narrow pulses. However, over the last five years, flavor and fragrance applications of
318 GC×GC with cryogenic-free thermal modulators based on differential-flow modulation (FM)
319 dynamics have been described.⁴⁷⁻⁴⁹

320 FM devices, based on the original design by Seeley *et al.*,⁵⁰ have a simple and effective
321 design, low operational and hardware costs, and high robustness. Interesting solutions in this field
322 have been introduced by Tranchida et al.^{49,51,52} and commercial products are nowadays available
323 from different manufacturers (e.g., Agilent Technologies, Sep Solve Analytical, and Chromaleont).
324 Cordero et al.⁵³ recently discussed the advantages of a new generation of FM devices
325 implemented with a Capillary Flow Technology (CFT) microfluidic plate and reverse fill/flush (RFF)
326 injection. The prototype, made available by Agilent is shown in the supplementary material
327 (Supplementary Figure SF4), has several advantages: (a) efficient band re-injection, (b) improved

328 ²D peak widths and symmetry, and (c) effective handling of collection-channel overloading.

329 Results on system performances (separation power, peak-widths, and separation space
330 used) were assessed for a model mixture of volatiles while medium complexity essential oils^{53,54}
331 (e.g., mint and lavender) were used to test profiling and quantitation accuracy with both external
332 calibration on the MS signals and predicted relative response factors (RRF) on the flame ionization
333 detector (FID) signals. Figure 5 shows the contour plots of peppermint and spearmint essential
334 oils. The enlarged areas show details of the elution regions of (A) menthols and (B) carvone
335 derivatives. For details on analysis conditions see the figure caption.

336 The system potentials for quantitative profiling were confirmed by accuracy results
337 obtained by cross-validating quantitation data from dual-parallel secondary columns and
338 detection. Identity confirmation and quantitation by MS signals completed the information
339 provided by FID that, in its turn, enabled extending quantitation to all identified components by
340 using FID-Predicted Retention Factors (PRFs)⁵⁵. Experimental results presented in this study,
341 together with the acceptable operational costs and relative ease of use and simple maintenance,
342 indicate that CFT reverse-inject differential flow modulation can contribute to promoting the use
343 of GC×GC for routine analysis in the flavor and fragrance field.

344

345 **Multidimensional data set elaboration challenges**

346 Multidimensional separation techniques enable effective insights into the composition of
347 complex samples. In particular, comprehensive GC offers unequalled information on samples
348 dimensionality by producing resolved and informative separation patterns, i.e., chemical
349 fingerprints with a great potential for comparative purposes. However, the data size and
350 complexity is challenging. Cross-sample studies in food aroma characterization have several
351 purposes: (a) sample classification *versus* sensory profile; (b) chemical fingerprinting to

352 characterize samples against reference standards; (c) progressive and time resolved monitoring of
353 chemical changes a function of technological/enzymatic treatments; and (d) discovering
354 informative markers for botanical/geographical assessment.

355 The most relevant *features* (i.e., chemical constituents characterized by relative position in
356 the chromatographic space and detector or mass spectral intensities) of a cross-sample analysis
357 are generally not known a priori and sometimes correspond to trace analytes. Informative data
358 elaboration therefore should combine non-targeted and targeted approaches to achieve the most
359 inclusive fingerprinting investigation.⁵⁶

360 Most of the studies in the field of aroma characterization by H/C MDGC and GC×GC adopt
361 targeted approaches: relevant analytes are first identified on the basis of their EI-MS spectrum
362 and relative retention ($^1D \bar{I}$'s) then their relative amounts are compared across samples for
363 classification and characterization. Interestingly, a bio-guided assay (e.g. GC-O) could preliminarily
364 target/tag odor informative retention regions driving targeted data elaboration in specific regions
365 of the chromatographic space.

366 In the authors' opinion, the most challenging aspect involves untargeted analysis by GC×GC
367 because it requires dedicated software(s) and skillful analysts and is in general under-exploited in
368 the field of aroma investigations.

369 In a study aimed at profiling volatiles from apple, pear, and quince fruits, Schmarr and
370 Bernhardt⁵⁷ analyzed 2D patterns by adapting a *peak-region feature* approach commonly used for
371 2D gel electrophoresis. The volatile fraction, sampled by HS-SPME, was analyzed with GC×GC-MS
372 to generate a 3D data matrix for each single analysis. The raw data then was converted to a
373 grayscale jpeg image by an open-source software (ImageJ™, Wayne Rasband, National Institute of
374 Health, USA) and processed with algorithms commonly used for 2D gel electrophoresis. The
375 approach included pre-processing operations (image alignment by warping and summation) that

376 produced a representative cumulative chromatogram (fusion image) of all of the constituents in all
377 samples. Figure 6 shows the complete workflow of the proposed method.

378 Boundaries around single 2D peaks were treated as regions in a template. The template
379 geometrically mapped back to each chromatogram and used to extract detector responses from
380 each chromatographic region, thus generating a data matrix of aligned regions for all samples.
381 Feature matching was, at that time, performed by retention-times mapping and visual
382 interpretation. As a constraint, at that time, MS data were not included for a direct verification of
383 alignments or for peak identification. Nowadays, an improved release of the original software is
384 available offering additional tools for spectra matching, visual comparison of chromatogram pairs,
385 and various post-processing possibilities including multivariate analysis (MVA) and chemometrics
386 (Decodon, Greifswald, Germany).

387 Smart Templates™ with peak-region features were developed by Reichenbach and co-
388 workers,⁵⁸ then implemented as a basic tool for comparative and explorative analysis in a
389 commercial software package (GC Image, Lincoln NE, USA) and used for targeted and untargeted
390 fingerprinting in different application fields. Very recently, a straightforward concept has been
391 exploited by combining targeted and untargeted approaches to obtain most comprehensive
392 results. The procedure, defined *UT fingerprinting* (i.e. *untargeted* and *targeted* fingerprinting), was
393 tested on extra-virgin olive oil (EVO oil) volatiles⁵⁶ and provided results for characterizing the
394 degree of olive ripening and EVO oil quality. Moreover, thanks to effective global transformation
395 algorithms^{59,60} for pattern re-alignment and template matching, a retrospective analysis was also
396 possible. This last option allowed retrospective re-evaluation of samples in light of new
397 informative features. Figures 7 show pseudocolor images that visualize the volatiles pattern from
398 EVO oils from two analytical campaigns within the three years of analysis. Figure 7A shows the 2D
399 chromatogram of a Spanish sample from 2015 harvest with an overlay of the template of peak-

400 regions adopted for cross-comparisons; Figure 7B shows the 2D chromatogram of an Italian EVO
401 oils sample (PDO Monti Iblei e Sicily Italy) analyzed in 2013. In the 2D patterns of these sample,
402 the peak-regions templates (light blue graphics and green circles) were matched after global
403 transformation of the original template to adapt the relative position of all peak-regions over the
404 2D pattern.^{60,61} This operation requires inspection by the analyst to check for relative
405 misalignments due to the column combination differences after two years of system operations,
406 but enables retrospective analysis of samples and re-consideration of analytical results.

407 EVO oils were the topic of an interesting study focused on sensory defects and their blue-
408 print within the volatiles mapped by GC×GC-MS. Purcaro et al.⁶² adopted the Smart Templates™
409 approach for an advanced fingerprinting investigation on olive oil samples, including reference
410 standards obtained from the International Olive Oil Council and commercial EVO oils. Samples,
411 submitted to sensory evaluation by an official panel prior to GC×GC analysis, were characterized
412 by targeted and untargeted approaches. A list of 261 reliably identified compounds was adopted
413 for the template and used to reveal the informative fingerprints related to the sensory
414 characteristics defined for each sample. These most informative compounds were collected in a
415 *blueprint* of specific defects (or combination of defects) adopted to discriminate *extra-virgin* from
416 defected oils (i.e. *lampante* oil) with the aid of a supervised approach, i.e. Partial Least Squares-
417 Discriminant Analysis (PLS-DA). The principle of sensomics, assigning higher information potential
418 to analytes with lower odor threshold, proved to be successful, and a much more powerful
419 discrimination of samples was obtained in view of a sensory quality assessment.

420 In the aroma analysis, multidimensional gas chromatographic techniques assumes a
421 valuable, sometimes indispensable, role (a) to study the composition of complex volatile fractions
422 very often consisting of hundreds of components (e.g., coffee, tea or cocoa) by profiling analysis,
423 (b) to detect key-odorants and explain their formation from precursors, (c) to discriminate

424 between enantiomer or coeluting (trace) components with different odor characteristics, and (d)
425 to understand the interaction/relationship with flavor perception, personal behavior, and health.
426 As extensively illustrated by the selected examples, MDGC is crucial to study the chemistry behind
427 sensory perception(s) since it offers high separation power and sensitivity that are fundamental
428 for accurate quantitation and to define informative fingerprints of complex samples to be
429 correlated with sensory qualities.

430 Crucial to MDGC advancement, in particular for GC×GC, is its link with sample preparation
431 and data elaboration, also in view of the development of a true “total analysis system”. The
432 present research trend goes towards the full on-line integration of sample preparation through
433 the adaption or introduction of new and/or dedicated techniques to make sampling a “true”
434 additional dimension of the analytical platform. At the same time, data elaboration is expected to
435 become the object of a radical evolution in the next years, as concurrently has happened in
436 metabolomics, again with an ever full integration with the analytical process. Effective and/but
437 operator-friendly processing tools enabling combined targeted and untargeted (fingerprinting)
438 investigations are desirable and expected especially when analytical data directly define peculiar
439 characteristic of the matrix.

440 **References**

- 441 (1) Hofmann T, Ottinger H, F. O. The taste activity concept: A powerful tool to trace the key
442 tastants in foods. *ACS Symp. Ser.* **2004**, *867*, 104–124.
- 443 (2) Charve, J.; Chen, C.; Hegeman, A. D.; Reineccius, G. A. Evaluation of instrumental methods
444 for the untargeted analysis of chemical stimuli of orange juice flavour. *Flavour Fragr. J.*
445 **2011**, *26* (6), 429–440.
- 446 (3) Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann,
447 T. Nature's chemical signatures in human olfaction: A foodborne perspective for future
448 biotechnology. *Angew. Chemie - Int. Ed.* **2014**, *53* (28), 7124–7143.
- 449 (4) Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models
450 and omission. *Chem. Senses* **2001**, *26* (5), 533–545.
- 451 (5) Cordero, C.; Kiefl, J.; Schieberle, P.; Reichenbach, S. E.; Bicchi, C. Comprehensive two-
452 dimensional gas chromatography and food sensory properties: Potential and challenges.
453 *Analytical and Bioanalytical Chemistry*. 2015, pp 169–191.
- 454 (6) Giddings, J. C. Sample dimensionality: A predictor of order-disorder in component peak
455 distribution in multidimensional separation. *J. Chromatogr. A* **1995**, *703* (1–2), 3–15.
- 456 (7) Lord, H. L.; Pfannkoch, E. A. *Sample preparation automation for gc injection*; Elsevier, 2012;
457 Vol. 2.
- 458 (8) Risticvic, S.; Vuckovic, D.; Lord, H. L.; Pawliszyn, J. 2.21 – Solid-Phase Microextraction. In
459 *Comprehensive Sampling and Sample Preparation*; 2012; pp 419–460.
- 460 (9) Full, Gerhard; Schreier, P. Covalent chromatography. A valuable method for the aroma
461 analysis of thiols at trace levels. *Lebensmittelchemie* **1994**, *48* (1), 1–4.
- 462 (10) Ross, C. F. *2.02 – Headspace Analysis*; Elsevier, 2012; Vol. 2.
- 463 (11) Bicchi, C.; Cordero, C.; Liberto, E.; Sgorbini, B.; Rubiolo, P. Headspace sampling of the

- 464 volatile fraction of vegetable matrices. *J. Chromatogr. A* **2008**, *1184* (1–2), 220–233.
- 465 (12) Bicchi, C.; Cordero, C.; Rubiolo, P. A survey on high-concentration-capability headspace
466 sampling techniques in the analysis of flavors and fragrances. *J. Chromatogr. Sci.* **2004**, *42*
467 (8).
- 468 (13) Kremser, A.; Jochmann, M. A.; Schmidt, T. C. PAL SPME Arrow - Evaluation of a novel solid-
469 phase microextraction device for freely dissolved PAHs in water. *Anal. Bioanal. Chem.* **2016**,
470 *408* (3), 943–952.
- 471 (14) Kremser, A.; Jochmann, M. A.; Schmidt, T. C. Systematic comparison of static and dynamic
472 headspace sampling techniques for gas chromatography. *Anal. Bioanal. Chem.* **2016**, 1–13.
- 473 (15) Helin, A.; Rönkkö, T.; Parshintsev, J.; Hartonen, K.; Schilling, B.; Läubli, T.; Riekkola, M. L.
474 Solid phase microextraction Arrow for the sampling of volatile amines in wastewater and
475 atmosphere. *J. Chromatogr. A* **2015**, *1426*, 56–63.
- 476 (16) Cordero, C.; Kiefl, J.; Schieberle, P.; Reichenbach, S. E.; Bicchi, C. Comprehensive two-
477 dimensional gas chromatography and food sensory properties: Potential and challenges.
478 *Analytical and Bioanalytical Chemistry*. Springer Verlag 2015, pp 169–191.
- 479 (17) Mastello, R. B.; Capobiango, M.; Chin, S.-T.; Monteiro, M.; Marriott, P. J. Identification of
480 odour-active compounds of pasteurised orange juice using multidimensional gas
481 chromatography techniques. *Food Res. Int.* **2015**, *75*, 281–288.
- 482 (18) Chin, S. T.; Eyres, G. T.; Marriott, P. J. Application of integrated
483 comprehensive/multidimensional gas chromatography with mass spectrometry and
484 olfactometry for aroma analysis in wine and coffee. *Food Chem.* **2015**, *185*, 355–361.
- 485 (19) Mitrevski, B.; Marriott, P. J. Novel hybrid comprehensive 2D - Multidimensional gas
486 chromatography for precise, high-resolution characterization of multicomponent samples.
487 *Anal. Chem.* **2012**, *84* (11), 4837–4843.

- 488 (20) Chin, S.-T.; Maikhunthod, B.; Marriott, P. J. Universal method for online enrichment of
489 target compounds in capillary gas chromatography using in-oven cryotrapping. *Anal. Chem.*
490 **2011**, *83* (17), 6485–6492.
- 491 (21) Chin, S.-T.; Eyres, G. T.; Marriott, P. J. System design for integrated comprehensive and
492 multidimensional gas chromatography with mass spectrometry and olfactometry. *Anal.*
493 *Chem.* **2012**, *84* (21), 9154–9162.
- 494 (22) Magagna, F.; Cordero, C.; Cagliero, C.; Liberto, E.; Rubiolo, P.; Sgorbini, B.; Bicchi, C. Black
495 tea volatiles fingerprinting by comprehensive two-dimensional gas chromatography - mass
496 spectrometry combined with high concentration capacity sample preparation techniques:
497 toward a fully automated sensomic assessment. *Food Chem.* **2017**, *225*, 276–287.
- 498 (23) Chin, S. T.; Eyres, G. T.; Marriott, P. J. Cumulative solid phase microextraction sampling for
499 gas chromatography-olfactometry of Shiraz wine. *J. Chromatogr. A* **2012**, *1255*, 221–227.
- 500 (24) Cordero, C.; Cagliero, C.; Liberto, E.; Nicolotti, L.; Rubiolo, P.; Sgorbini, B.; Bicchi, C. High
501 concentration capacity sample preparation techniques to improve the informative potential
502 of two-dimensional comprehensive gas chromatography-mass spectrometry: Application to
503 sensomics. *J. Chromatogr. A* **2013**, *1318*, 1–11.
- 504 (25) Marriott, P. J.; Chin, S. T.; Maikhunthod, B.; Schmarr, H. G.; Bieri, S. Multidimensional gas
505 chromatography. *TrAC - Trends in Analytical Chemistry*. 2012, pp 1–20.
- 506 (26) Legrum, C.; Slabizki, P.; Schmarr, H.-G. Enantiodifferentiation of 3-sec-butyl-2-
507 methoxypyrazine in different species using multidimensional and comprehensive two-
508 dimensional gas chromatographic approaches. *Anal. Bioanal. Chem.* **2015**, *407* (1), 253–263.
- 509 (27) Legrum, C.; Gracia-Moreno, E.; Lopez, R.; Potouridis, T.; Langen, J.; Slabizki, P.; Weiland, J.;
510 Schmarr, H. G. Quantitative analysis of 3-alkyl-2-methoxypyrazines in German Sauvignon
511 blanc wines by MDGC-MS or MDGC-MS/MS for viticultural and enological studies. *Eur. Food*

- 512 *Res. Technol.* **2014**, 239 (4), 549–558.
- 513 (28) Welke, J. E.; Zanus, M.; Lazzarotto, M.; Alcaraz Zini, C. Quantitative analysis of headspace
514 volatile compounds using comprehensive two-dimensional gas chromatography and their
515 contribution to the aroma of Chardonnay wine. *Food Res. Int.* **2014**, 59, 85–99.
- 516 (29) Imelda, R.; Pan, B. S.; Sacks, G. L. Rapid measurement of 3-Alkyl-2-methoxypyrazine content
517 of winegrapes to predict levels in resultant wines. *J. Agric. Food Chem.* **2009**, 57 (18), 8250–
518 8257.
- 519 (30) Schmarr, H.-G.; Ganss, S.; Koschinski, S.; Fischer, U.; Riehle, C.; Kinnart, J.; Potouridis, T.;
520 Kuttyrev, M. Pitfalls encountered during quantitative determination of 3-alkyl-2-
521 methoxypyrazines in grape must and wine using gas chromatography–mass spectrometry
522 with stable isotope dilution analysis. Comprehensive two-dimensional gas chromatography–
523 mass spectrometr. *J. Chromatogr. A* **2010**, 1217 (43), 6769–6777.
- 524 (31) Schmarr, H. G.; Slabizki, P.; Müntnich, S.; Metzger, C.; Gracia-Moreno, E. Ionic liquids as
525 novel stationary phases in gas liquid chromatography: Inverse or normal isotope effect? *J.*
526 *Chromatogr. A* **2012**, 1270, 310–317.
- 527 (32) Schmarr, H. G.; Slabizki, P.; Legrum, C. Optimization in multidimensional gas
528 chromatography applying quantitative analysis via a stable isotope dilution assay. *Anal.*
529 *Bioanal. Chem.* **2013**, 405 (20), 6589–6593.
- 530 (33) Langen, J.; Wegmann-Herr, P.; Schmarr, H.-G. Quantitative determination of α -ionone, β -
531 ionone, and β -damascenone and enantiodifferentiation of α -ionone in wine for authenticity
532 control using multidimensional gas chromatography with tandem mass spectrometric
533 detection. *Anal. Bioanal. Chem.* **2016**, 408 (23), 6483–6496.
- 534 (34) Pineau, B.; Barbe, J.-C.; Van Leeuwen, C.; Dubourdieu, D. Which impact for beta-
535 damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, 55 (10), 4103–4108.

- 536 (35) Schmarr, H.-G.; Keiser, J.; Krautwald, S. An improved method for the analysis of 2-
537 aminoacetophenone in wine based on headspace solid-phase microextraction and heart-cut
538 multidimensional gas chromatography with selective detection by tandem mass
539 spectrometry. *J. Chromatogr. A* **2016**, *1477*, 64–69.
- 540 (36) Amirav, A.; Gordin, A.; Poliak, M.; Fialkov, A. B. Gas chromatography-mass spectrometry
541 with supersonic molecular beams. *J. mass Spectrom. JMS* **2008**, *43*, 141–163.
- 542 (37) Alam, M. S.; Stark, C.; Harrison, R. M. Using Variable Ionization Energy Time-of-Flight Mass
543 Spectrometry with Comprehensive GC×GC to Identify Isomeric Species. *Anal. Chem.* **2016**,
544 *88* (8), 4211–4220.
- 545 (38) H.-G. Schmarr, P. Slabizki, C. Legrum, J. Langen. Wenn Nanogramm das Aroma verändern.
546 *Nachr. Chem.* **2014**, *62*, 1192–1196.
- 547 (39) Liu, Z.; Phillips, J. B. Comprehensive two-dimensional gas chromatography using an on-
548 column thermal modulator interface. *J. Chromatogr. Sci.* **1991**, *29* (5), 227–231.
- 549 (40) Adahchour, M.; Beens, J.; Vreuls, R. J. J.; Batenburg, A. M.; Rosing, E. A. E.; Brinkman, U. A.
550 T. Application of solid-phase micro-extraction and comprehensive two-dimensional gas
551 chromatography (GC x GC) for flavour analysis. *Chromatographia* **2002**, *55* (5–6), 361–367.
- 552 (41) Adahchour, M.; van Stee, L. L. P.; Beens, J.; Vreuls, R. J. J.; Batenburg, M. A.; Brinkman, U. A.
553 T. Comprehensive two-dimensional gas chromatography with time-of-flight mass
554 spectrometric detection for the trace analysis of flavour compounds in food. *J. Chromatogr.*
555 *A* **2003**, *1019* (1–2), 157–172.
- 556 (42) Tranchida, P. Q.; Purcaro, G.; Maimone, M.; Mondello, L. Impact of comprehensive two-
557 dimensional gas chromatography with mass spectrometry on food analysis. *J. Sep. Sci.* **2015**,
558 *39* (1), n/a-n/a.
- 559 (43) Chin, S.-T.; Marriott, P. J. Multidimensional gas chromatography beyond simple volatiles

- 560 separation. *Chem. Commun.* **2014**, 50(64), 8819–8833.
- 561 (44) Chin, S. T.; Marriott, P. J. Review of the role and methodology of high resolution approaches
562 in aroma analysis. *Anal. Chim. Acta* **2015**, 854, 1–12.
- 563 (45) Chin, S. T.; Eyres, G. T.; Marriott, P. J. System design for integrated comprehensive and
564 multidimensional gas chromatography with mass spectrometry and olfactometry. *Anal.*
565 *Chem.* **2012**, 84 (21), 9154–9162.
- 566 (46) Maikhunthod, B.; Morrison, P. D.; Small, D. M.; Marriott, P. J. Development of a switchable
567 multidimensional/comprehensive two-dimensional gas chromatographic analytical system.
568 *J. Chromatogr. A* **2010**, 1217 (9), 1522–1529.
- 569 (47) Bernal, J.; Manzano, P.; Diego, J. C.; Bernal, J. L.; Nozal, M. J. Comprehensive two-
570 dimensional gas chromatography coupled with static headspace sampling to analyze volatile
571 compounds: Application to almonds. *J. Sep. Sci.* **2014**, 37 (6), 675–683.
- 572 (48) Manzano, P.; Arnáiz, E.; Diego, J. C.; Toribio, L.; García-Viguera, C.; Bernal, J. L.; Bernal, J.
573 Comprehensive two-dimensional gas chromatography with capillary flow modulation to
574 separate FAME isomers. *J. Chromatogr. A* **2011**, 1218 (30), 4952–4959.
- 575 (49) Tranchida, P. Q.; Maimone, M.; Franchina, F. A.; Bjerck, T. R.; Zini, C. A.; Purcaro, G.;
576 Mondello, L. Four-stage (low-)flow modulation comprehensive gas chromatography-
577 quadrupole mass spectrometry for the determination of recently-highlighted cosmetic
578 allergens. *J. Chromatogr. A* **2016**, 1439, 144–151.
- 579 (50) Bueno, P. A.; Seeley, J. V. Flow-switching device for comprehensive two-dimensional gas
580 chromatography. In *Journal of Chromatography A*; 2004; Vol. 1027, pp 3–10.
- 581 (51) Tranchida, P. Q.; Franchina, F. A.; Dugo, P.; Mondello, L. Flow-modulation low-pressure
582 comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **2014**, 1372, 236–
583 244.

- 584 (52) Tranchida, P. Q.; Franchina, F. A.; Dugo, P.; Mondello, L. Use of greatly-reduced gas flows in
585 flow-modulated comprehensive two-dimensional gas chromatography-mass spectrometry.
586 *J. Chromatogr. A* **2014**, *1359*, 271–276.
- 587 (53) Cordero, C.; Rubiolo, P.; Cobelli, L.; Stani, G.; Miliazza, A.; Giardina, M.; Firor, R.; Bicchi, C.
588 Potential of the reversed-inject differential flow modulator for comprehensive two-
589 dimensional gas chromatography in the quantitative profiling and fingerprinting of essential
590 oils of different complexity. *J. Chromatogr. A* **2015**, *1417*, 79–95.
- 591 (54) Cordero, C.; Rubiolo, P.; Reichenbach, S. E.; Carretta, A.; Cobelli, L.; Giardina, M.; Bicchi, C.
592 Method translation and full metadata transfer from thermal to differential flow modulated
593 comprehensive two dimensional gas chromatography: Profiling of suspected fragrance
594 allergens. *J. Chromatogr. A* **2016**.
- 595 (55) De Saint Laumer, J. Y.; Cicchetti, E.; Merle, P.; Egger, J.; Chaintreau, A. Quantification in gas
596 chromatography: Prediction of flame ionization detector response factors from combustion
597 enthalpies and molecular structures. *Anal. Chem.* **2010**, *82* (15), 6457–6462.
- 598 (56) Magagna, F.; Valverde-Som, L.; Ruíz-Samblás, C.; Cuadros-Rodríguez, L.; Reichenbach, S. E.;
599 Bicchi, C.; Cordero, C. Combined Untargeted and Targeted fingerprinting with
600 comprehensive two-dimensional chromatography for volatiles and ripening indicators in
601 olive oil. *Anal. Chim. Acta* **2016**, *936*.
- 602 (57) Schmarr, H.-G.; Bernhardt, J. Profiling analysis of volatile compounds from fruits using
603 comprehensive two-dimensional gas chromatography and image processing techniques. *J.*
604 *Chromatogr. A* **2010**, *1217* (4), 565–574.
- 605 (58) Cordero, C.; Liberto, E.; Bicchi, C.; Rubiolo, P.; Reichenbach, S. E.; Tian, X.; Tao, Q. Targeted
606 and non-targeted approaches for complex natural sample profiling by GCxGC-qMS. *J.*
607 *Chromatogr. Sci.* **2010**, *48* (4), 251–261.

- 608 (59) Reichenbach, S. E.; Rempe, D. W.; Tao, Q.; Bressanello, D.; Liberto, E.; Bicchi, C.; Balducci, S.;
609 Cordero, C. Alignment for Comprehensive Two-Dimensional Gas Chromatography with Dual
610 Secondary Columns and Detectors. *Anal. Chem.* **2015**, *87* (19), 10056–10063.
- 611 (60) Rempe, D. W.; Reichenbach, S. E.; Tao, Q.; Cordero, C.; Rathbun, W. E.; Zini, C. A.
612 Effectiveness of Global, Low-Degree Polynomial Transformations for GCxGC Data Alignment.
613 *Anal. Chem.* **2016**, acs.analchem.6b02254.
- 614 (61) Reichenbach, S. E.; Rempe, D. W.; Tao, Q.; Bressanello, D.; Liberto, E.; Bicchi, C.; Balducci, S.;
615 Cordero, C. Alignment for Comprehensive Two-Dimensional Gas Chromatography with Dual
616 Secondary Columns and Detectors. *Anal. Chem.* **2015**, *87* (19), 10056–10063.
- 617 (62) Purcaro, G.; Cordero, C.; Liberto, E.; Bicchi, C.; Conte, L. S. Toward a definition of blueprint
618 of virgin olive oil by comprehensive two-dimensional gas chromatography. *J. Chromatogr. A*
619 **2014**, *1334*, 101–111.
- 620
- 621

622 **Figures captions**

623 **Figure 1:** The Arrow SPME system with sorbent exposed (left) and with sorbent covered by a steel
624 tube (right).[From Helin *et al*¹⁵.]

625 **Figure 2:** Configuration for the H/C MDGC-MS/MS system, as reported by Legrum *et al.*¹⁸, with AC
626 (analytical column), RC (restriction capillary), EPC (electronic pressure control), and QqQ (triple
627 quadrupole) MS.

628 **Figure 3:** H/C MDGC chromatograms (²D) showing co-elution in a real wine sample (Pinot Noir)
629 during the analysis of α -ionone depending on selection of SRM transitions (from Langen *et al.*³³).
630 Analytical columns: ¹D column (30m×0.25 mm i.d. ×0.25 μ m of polyethylene glycol (StabilWax-MS,
631 Restek, Bad Homburg, Germany); ²D column (25 m×0.25 mm i.d. Lipodex G[®] - octakis(2,3-di-*O*-
632 pentyl-6-*O*-methyl)- γ -cyclodextrin, Macherey-Nagel). Carrier gas: helium in constant pressure. H/C
633 and Oven temperature conditions are detailed in the reference paper by Langen *et al.*³³.

634 **Figure 4: (4A)** Pseudocolored GC×GC chromatogram of the volatile fraction of an Extra Virgin
635 Olive oil from the Granada region (Spain). **(4B)** Positions of 119 known target peaks (empty light
636 green circles) linked by red lines to the ISTD α -tujone (black circle). **(4C)** Retention area of highly
637 volatile compounds in the white rectangle of Figure 6A. [From Magagna *et al.*⁵⁶]

638 GC×GC-MS analyses used: ¹D column (30 m×0.25 mm i.d. ×0.25 μ m of polyethylene glycol (Solgel-
639 Wax; SGE, Ringwood, Australia); ²D column (1 m×0.10 mm i.d. × 0.1 μ m of 86%
640 polydimethylsiloxane, 7% phenyl, 7% cyanopropyl (OV1701, Mega, Legnano, Italy). MS was a fast
641 scanning single quadrupole operating at 12,500 amu/s; scan range 40-240 *m/z*, acquisition
642 frequency 28 Hz. Carrier gas: helium in constant flow. Modulation parameters and oven
643 temperature conditions are detailed in the reference paper. Volatiles were extracted by HS-SPME
644 from 1.500 g of EVO oil in a 20 mL glass vial; sampling time 40 min at 50°C. SPME fiber was a
645 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μ m, 2 cm length

646 stableflex fiber from Supelco (Bellefonte, PA, USA).

647 **Figure 5:** Pseudocolor images of a peppermint and of a spearmint essential oil. Enlarged areas
648 show details of the elution of (A) menthols and (B) carvone derivatives. [From Cordero *et al.*⁵³]
649 GC×GC-MS analysis with reverse-inject differential flow modulation used: ¹D column (10 m×0.10
650 mm i.d. ×0.10 μm of polyethylene glycol (Solgel-Wax; SGE, Ringwood, Australia); two parallel ²D
651 columns (1.5 m×0.10 mm i.d. × 0.1 μm of 86% polydimethylsiloxane, 7% phenyl, 7% cyanopropyl
652 (OV1701, Mega, Legnano, Italy). Detection was by parallel FID/MS; MS was a fast scanning single
653 quadrupole operating at 12,500 amu/s; scan range 40-240 *m/z*, acquisition frequency 28 Hz.
654 Carrier gas: helium. Modulation parameters and oven temperature conditions are detailed in the
655 reference paper.

656 **Figure 6:** Workflow from Schmarr and Bernhardt⁵⁷: (1) Samples prepared and analyzed by HS-
657 SPME-GC×GC-qMS; (2) 2D GC chromatograms transformed into 32-bit images; (3) 2D GC images
658 stored in Delta2D™ software; (4) Positional correction (warp vectors) for image congruency (dual
659 channel overlay color code: blue = image1, orange = image2, and black = overlap); (5) Volatiles
660 map from project-wide 2D GC image fusion; (6) Detected spot consensus; (7) Spot consensus
661 boundaries applied to all 2D GC images for gray level integration; (8) Gray level integration results
662 in quantitative data which can be summarized in volatile profiles (blue – low amount, black –
663 average amount, orange – large amount of volatile).

664 **Figure 7:** Contour plots of the volatiles pattern from EVO oils from two analytical campaigns within
665 a three years of analysis. [From Magagna *et al.*⁵⁶] **(7A)** Spanish sample from 2015 harvest with an
666 overlay of the template of *peak-regions* (light blue graphics) adopted for cross-comparisons; **(7B)**
667 Italian EVO oils sample (PDO Monti Iblei e Sicily Italy) analyzed in 2013 with an overlay of the *peak-*
668 *regions* template from **7A** after matching and global transformation. For analytical conditions, see
669 the caption of Figure 4.

670 **Associated content**

671 **Supplementary Figure SF1:** *Brettanomyces* off-flavor compounds in a red wine after HS-SPME and
672 GC×GC-TofMS analysis. The enlarged area shows the increased selectivity when using molecular
673 ions for generating an extracted ion 3D plot facilitating identification of (A) 4-ethylguaiacol and (B)
674 4-ethylphenol. [From Schmarr *et al.* ³⁸]

675

676 **Supplementary Figure SF2:** Hi-res MS as a powerful tool for structure elucidation, here for
677 identifying the structure of a common fragment ion ($m/z = 121$) in the spectra of β -damascenone
678 and α -ionone (these can be retrieved at <http://webbook.nist.gov/chemistry>).

679

680 **Supplementary Figure SF3:** Instrument schematic of the integrated GC×GC/GC–GC system
681 with flame ionisation, olfactory and mass spectral detections. [From Chin *et al.* ⁴³]

682

683 **Supplementary Figure SF4:** Schematic diagram of the reverse-inject differential flow modulator
684 prototyped by Agilent in loading state (A) and injection state (B). [From Cordero *et al.* ⁵³]

685

686

Figure 1

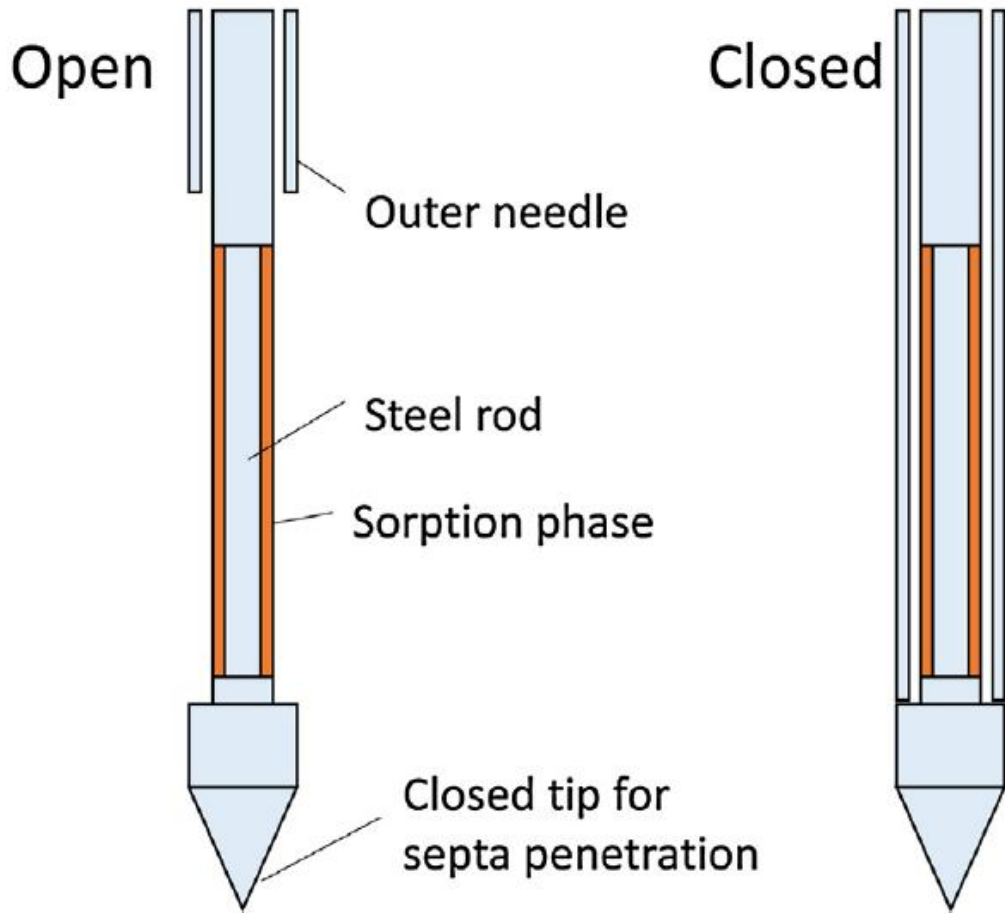


Figure 2

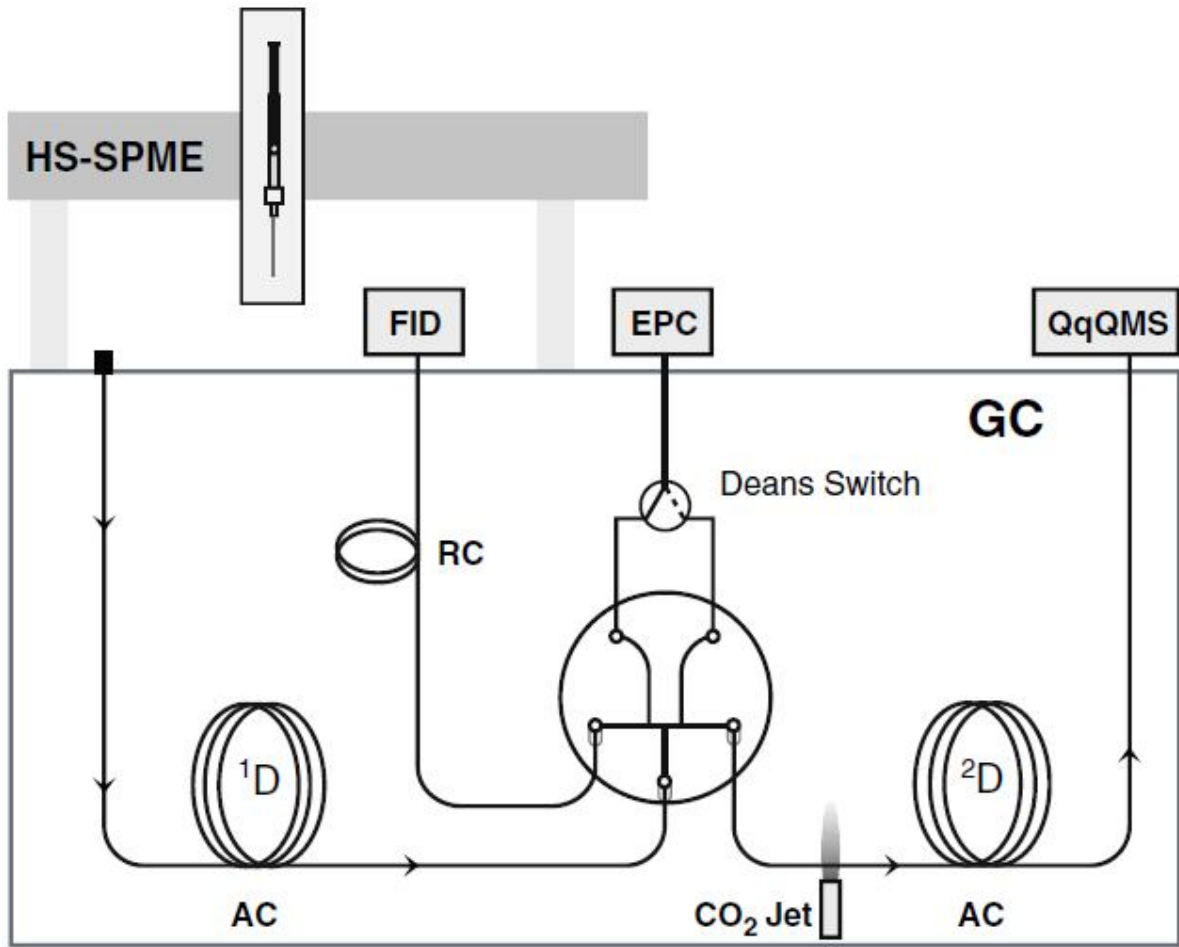


Figure 3

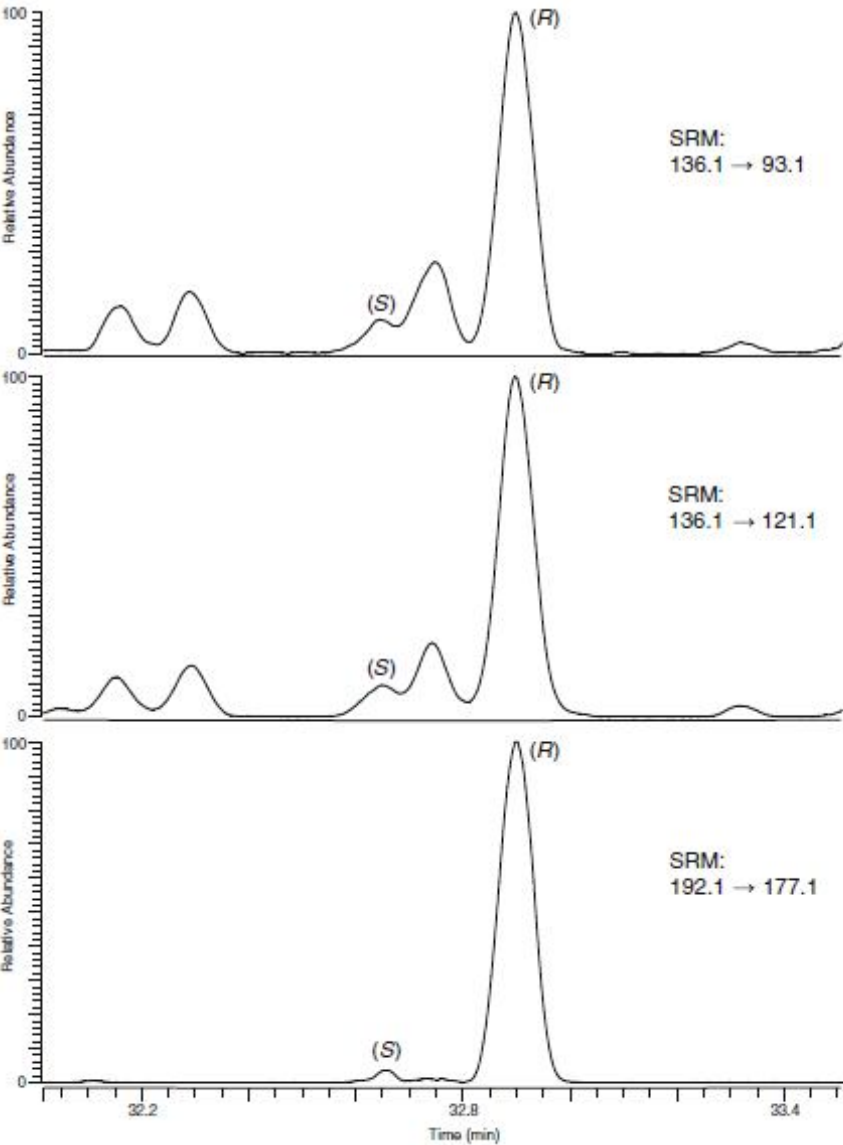


Figure 4

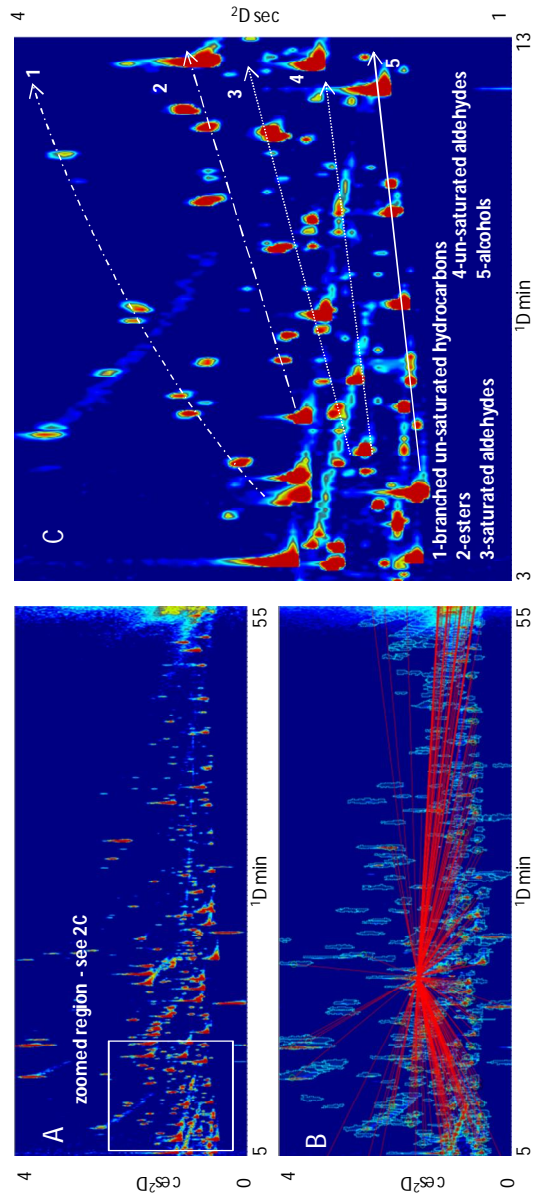


Figure 5

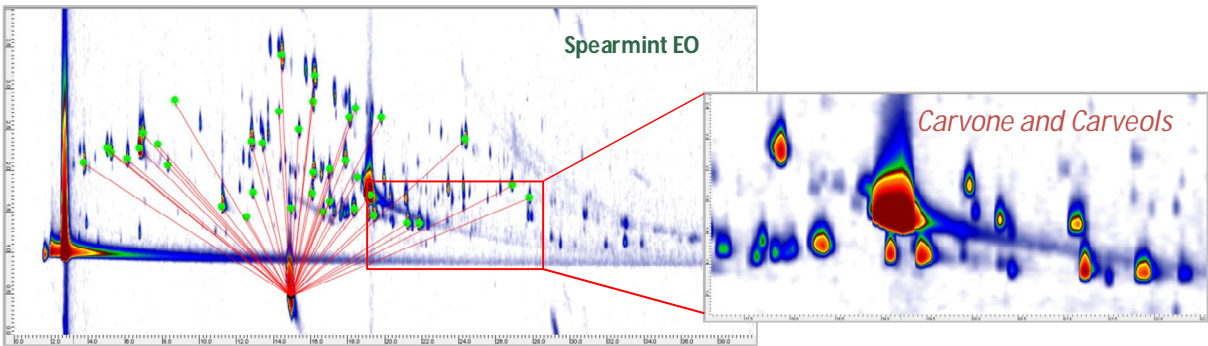
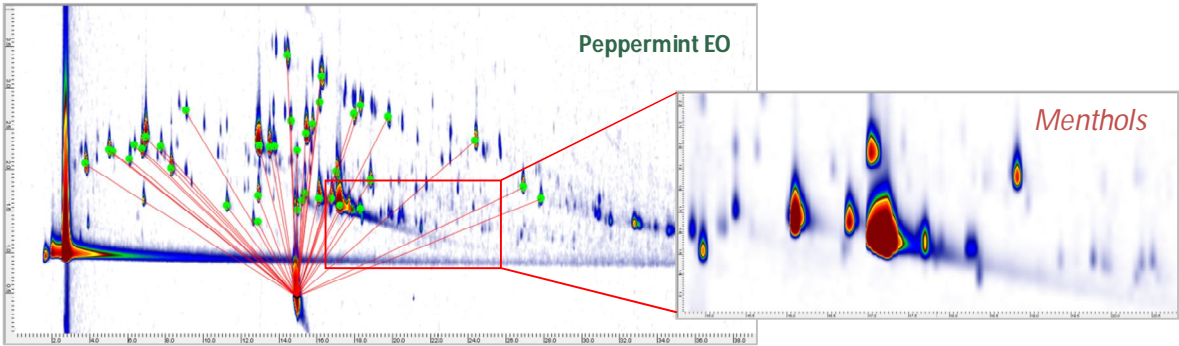


Figure 6

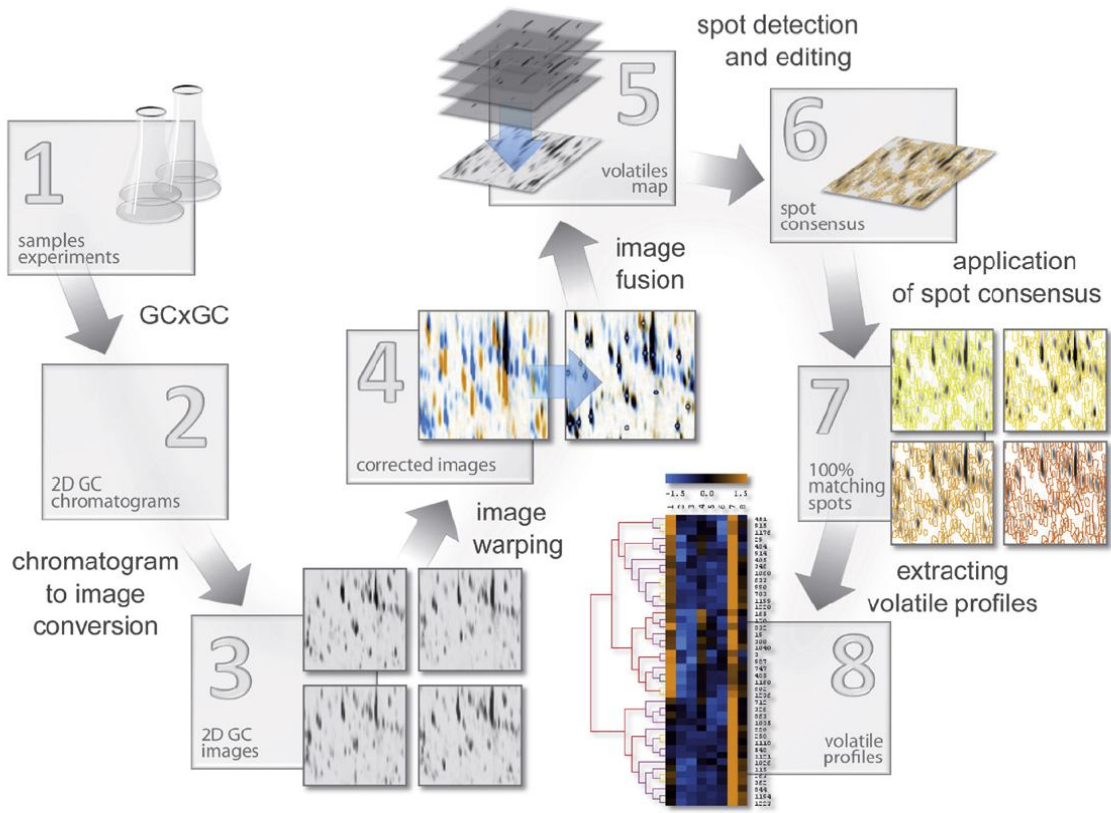
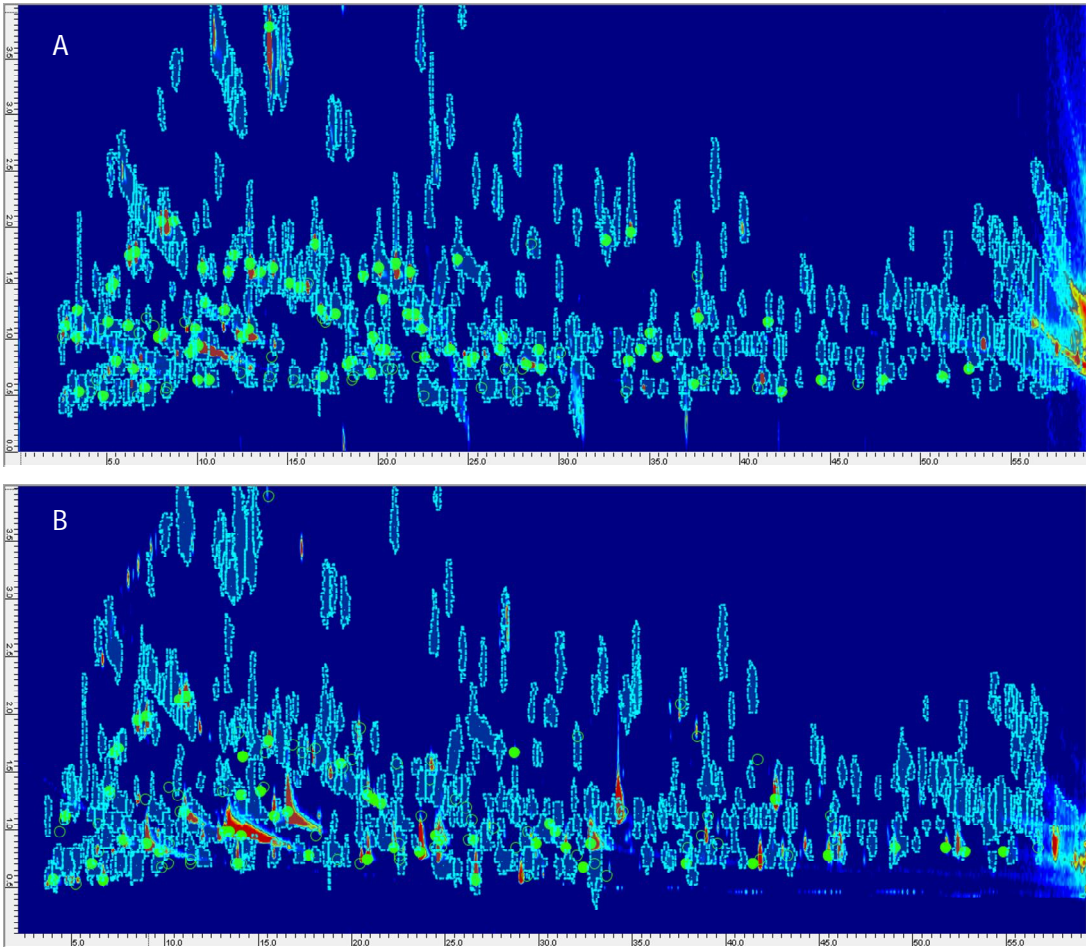


Figure 7



TOC graphic

