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Parallel dual secondary-column-dual detection comprehensive two-dimensional gas chromatography: a flexible and reliable analytical tool for essential oils quantitative profiling

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1 **Parallel dual secondary-column-dual detection comprehensive two-dimensional**
2 **gas chromatography: a flexible and reliable analytical tool for essential oils**
3 **quantitative profiling.**

4

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21 **Abstract**

22 Comprehensive two-dimensional gas chromatography (GC×GC) coupled with mass spectrometry (MS) is
23 one of the most powerful analytical techniques now available for detailed analysis, identification, and
24 quantitation of medium-to-high complexity mixtures. However the number of application methods that
25 combine fingerprinting and/or profiling with quantitation of informative volatile analytes (targets) is still
26 limited. Possible reasons are related to the huge amount of information to handle and to the availability of
27 reference standards for calibration. Although quantitative analysis by GC×GC is complex it has important
28 advantages: (a) to assess data/results over an extended time frame, varied instrumentation, and different
29 laboratories; (b) to interpret the biological role of (potential) biomarkers; (c) to evaluate the impact of
30 potent odorants; and/or (d) to define product quality, *e.g.*, relative to a reference standard.

31 In this study, a GC×2GC-MS/FID platform consisting of one primary column (¹D) coupled to two parallel
32 secondary columns (²D) having identical inner diameter, stationary phase chemistry, and film thickness,
33 which, in turn, are connected to two detectors: a fast quadrupole MS and a FID, was adopted for
34 quantitative profiling of essential oils (EOs).

35 Two medium complexity EOs (*i.e.*, *Mentha* and *Lavandula* species) that pose different quantitation
36 challenges were taken as examples and a selection of quality markers subjected to an extensive method
37 performance evaluation (*e.g.*, method validation). Experimental results confirmed the platform's reliability
38 in terms of: linearity, precision, and quantitation accuracy. In addition, predicted FID Relative Response
39 Factors (RRFs) based on combustion enthalpies were adopted to extend quantitation to all identified
40 analytes. The experimental data demonstrated the accuracy of the predicted RRFs, supporting their
41 adoption in quantitation of EO markers. This approach is of particular interest for those applications where
42 reference standards are not (easily) available and/or regulated.

43

44

45

46 **Key-words:**

47 Comprehensive two-dimensional gas chromatography-mass spectrometry; parallel dual secondary column-
48 dual detection; essential oil analysis; global quantitative profiling; Predicted Relative Response Factors

49

50 Introduction

51 Comprehensive two-dimensional gas chromatography (GC×GC) coupled with mass spectrometry (MS) is
52 one of the most powerful analytical techniques now available for detailed analysis, identification, and
53 quantitation of medium-to-high complexity mixtures. Compared to one-dimensional systems, GC×GC
54 applies different selectivity in two chromatographic dimensions to provide higher separation power and
55 unmatched peak capacity [1,2] combined with meaningful 2D elution patterns that facilitate analyte
56 identification and sample fingerprinting. However, as reviewed by Marriott *et al.* [3] and more recently by
57 Cordero *et al.* [4], the number of application methods that combine fingerprinting and profiling with
58 quantitation of informative volatile analytes (targets) is still limited. A possible reason is that by increasing
59 the number of analytes subjected to quantitation, the nature and amount of information to handle
60 exponentially increases. The validation process for multi-analyte quantitative methods requires intensive
61 data elaboration and extensive automation; otherwise, the benefits of single-run quantitation with GC×GC
62 could be compromised by cumbersome and time-consuming data treatment. An example is linearity
63 assessment, for which multi-analyte calibration operations must cover multiple concentration ranges
64 (which sometimes are not contiguous), so accuracy should be validated over a wide range of
65 concentrations where the matrix can differently exert detrimental effects on quantitation.

66 Although quantitative analysis by GC×GC is complex and time-consuming, it has important advantages,
67 even when not mandatory [5,6]: (a) to assess data/results over an extended time frame, varied
68 instrumentation, and different laboratories; (b) to interpret the biological role of (potential) biomarkers [7];
69 (c) to evaluate the impact of potent odorants on overall food aroma [8]; and/or (d) to define product
70 quality, *e.g.*, relative to a reference standard.

71 For complex samples which may exhibit hundreds of potentially informative peaks, as is the case for some
72 essential oils (EOs), the possibility of an extended/full quantitative assessment is attractive. However,
73 quantitation by external standard calibration or by Relative Response Factors (*RRFs*) [9-13] may not be
74 practical because of: (a) the lack of authentic/reference standards, and (b) the time required for multiple-
75 compound calibration (as discussed above).

76 Most quantitative methods validated for targeted analysis by GC×GC are based on MS detection (high
77 frequency Time-of-Flight MS (ToFMS) or fast quadrupole MS (qMS)) because this approach commonly is
78 mandatory for regulated substances (xenobiotics, residues, contaminants, suspected allergens, *etc.*). In
79 addition, for trace and ultra-trace analytes, as with several aroma compounds present in a food at sub-
80 mg/Kg level [9,14,15], with MS detection, diagnostic ions can be used to increase Signal-to-Noise (S/N)
81 ratios and thus method sensitivity.

82 In the EOs analysis, the challenges of quantifying the large number of peaks generated by GC×GC can be
83 overcome, for non-regulated analytes in sample quality assessment, by adopting Predicted Flame Ionization
84 Detector (FID) *RRFs* based on combustion enthalpies and molecular structure. This approach enables

85 quantitation without external standards. It was introduced by de Saint Laumer *et al.* [16] and applied to
86 different quantitation problems by Tissot *et al.* [17], in particular for quantitation of markers of bergamot
87 EO, purity assessment of unstable or reactive analytes, and profiling of dynamic mixtures stored at different
88 temperature and pH conditions.

89 Predicted FID RRFs have been validated for GC×GC-FID applications by quantifying model mixtures of
90 interest in the fragrance field by Tissot *et al.* [17] and, more recently, by Filippi *et al.* [18] for characterizing
91 vetiver EOs from different geographical origins by GC×GC-MS and GC×GC-FID.

92 Because of the complementary attributes of FID responses and MS information, a GC×GC analytical
93 platform that employs both types of detectors has exciting potential and so is of great interest. The
94 concurrent presence of the two detectors operating on different principles, not only provides data for
95 simultaneous analyte identification and quantitation, but also facilitates internal cross-validation of results
96 [7]. The alignment of the separation patterns obtained with the two detectors at the data elaboration level
97 allows unified consideration of the resulting data set from the integrated system.

98 A GC×2GC-MS/FID platform therefore has been implemented, inspired by previous papers from other
99 researchers [18,19,20] with one primary column (¹D) coupled to two parallel secondary columns (²D) having
100 identical inner diameter, stationary phase chemistry, and film thickness, which, in turn, are connected to
101 two detectors: a fast quadrupole MS and a FID [22]. Cryotrapping and refocusing is achieved with a dual-
102 stage, loop-type thermal modulator at the head of the ²D columns.

103 Unlike previous GC×GC platforms with a single ²D column and dual parallel detection (MS/FID or MS/SCD),
104 the adoption of two parallel secondary columns enables the system to operate at closer-to-optimal ²D
105 linear velocities and to double ²D loading capacity, with positive effects on overall system orthogonality,
106 resolution, and peak capacity. This last characteristic is fundamental when FID detection is used for
107 quantitation purposes. With FID, quantitative accuracy requires highly resolved peaks; co-elutions generate
108 quantitation errors that can be solved by adding a further system informative dimension, as that provided
109 by MS. Previous studies on model solutions (homologue series of linear hydrocarbons and suspected
110 volatile allergens) and on a medium-complexity EO (*Artemisia umbelliformis* Lam.) indicated that the
111 GC×2GC-MS/FID provides consistent results, both in terms of analyte identifications (*e.g.*, reliability of
112 spectra and MS matching) and the peaks' quantitative descriptors (*e.g.*, number of scans-per-peak and
113 precision) [22].

114 In the present study, the GC×2GC-MS/FID platform is subjected to an extensive performance evaluation,
115 with a focus on the method's accuracy and quantitation reliability, to evaluate its potential for the
116 quantitative profiling of EOs. Two medium complexity EOs (*i.e.*, *Mentha* and *Lavandula* species) have been
117 taken as examples that pose different quantitation challenges. *Mentha* spp. EOs were selected because of
118 the presence of high abundance components (30-70 g/100g) closely eluting to informative quality markers
119 that are present at low concentrations (0.1 g/100g), thus interfering with correct multi-target quantitation.

120 In addition, quantitative assessment of *Mentha* spp. EOs by 1D-GC-MS and GC-FID in a previous study [12]
121 provides reference data for the current method validation and for more reliable evaluation of the system's
122 potential. On the other hand, *Lavandula* spp. EOs were chosen because of the challenging quantitation
123 aspects related to the complexity of the 2D patterns generated by adding a further informative dimension
124 to the analytical platform, *i.e.*, the chiral recognition. The GC×2GC-MS/FID platform, in this case, is
125 implemented by a ¹D Enantio-Selective (ES) stationary phase.

126 Target analytes included in the method validation process have been selected because of their role as
127 markers for quality and/or botanical origin assessment. It is noteworthy that some of the selected analytes
128 also recently have been mentioned in the Official Opinion of the EU Scientific Committee on Consumer
129 Safety on "Fragrance allergens in cosmetic products" [23] and in a near future should be accurately
130 quantified in cosmetics and/or fragrances to comply with regulatory requirements. Validation is focused on
131 method linearity, precision, and accuracy against authentic reference standards. In addition, method
132 suitability for full/extensive quantitation based on FID Predicted RRFs is verified.

133

134 **Experimental**

135 **Essential Oils (EO) samples, reference standards for calibration and solvents**

136 Pure standards of *n*-alkanes (from *n*-C₉ to *n*-C₂₅) for Linear Retention Indices (I^T_s) calibration, Internal
137 Standard (ISTD) calibration, and Internal Quality Control (QC) verification were from Sigma-Aldrich (Milan,
138 Italy).

139 Pure standards of 1,8-cineole, (*R*)-(+)-limonene, linalool, camphor, (+)-isopulegol, (-)-menthone, (+)-
140 menthofuran, (+)-neomenthol, (2*S*)- (+)-borneol, lavandulol, menthol, (*S*)-(+)-4-terpineol, (1*S*,2*R*,5*R*)-(+)-
141 isomenthol, (*R*)-(+)-pulegone, (*R*)-(-)-carvone, linalyl acetate, and menthyl acetate were from Sigma-Aldrich
142 (Milan, Italy). (*R*)-(-)-lavandulyl acetate and (+)-neoisomenthol were from authors' laboratory.

143 Solvents (cyclohexane and dichloromethane) were all HPLC-grade, from Sigma-Aldrich (Milan, Italy).

144 *Mentha x piperita* L. EO (CS PEPP) was prepared in agreement to the method of the European
145 Pharmacopoeia [24] and kindly supplied by Dr. Franco Chialva (ChialvaMenta, Pancalieri, Turin Italy).

146 Commercial samples (CS) of *Mentha arvensis* L. (CS ARV), *Mentha spicata* L. (CS SPEAR), and *Mentha x*
147 *gentilis* L. (CS GENT) EOs were purchased from the market.

148 *Lavandula angustifolia* Mill. (CS LAV01, CS LAV02, and CS LAV03) and *Lavandula angustifolia* Mill. x
149 *Lavandula latifolia* Medik (lavandin Grosso) (CS GROSS) EOs were purchased from the market.

150

151 **Calibration solutions and EO samples dilutions**

152 Standard Stock Solutions of reference analytes were prepared at a concentration of 10 mg/mL in
153 dichloromethane and stored at -18°C.

154 Calibration solutions for *Mentha* spp. EOs quantitation were prepared in dichloromethane by mixing
155 suitable volumes of single component Standard Stock Solutions of 1,8-cineole, (*R*)-(+)-limonene, linalool,
156 (+)-isopulegol, (-)-menthone, (+)-menthofuran, (+)-neomenthol, menthol, 4-terpineol, (1*S*,2*R*,5*R*)-(+)-
157 isomenthol, (+)-neoisomenthol, (*R*)-(+)-pulegone, (*R*)-(-)-carvone, menthyl acetate, *n*-decane, *n*-undecane,
158 *n*-dodecane, and *n*-tridecane. ISTD (*n*-Pentadecane) was included at a concentration of 25 mg/L.

159 Calibration solutions for *Lavandula* spp. EOs quantitation were prepared in cyclohexane by mixing suitable
160 volumes of single component Standard Stock Solutions of (*R*)-(+)-Limonene, 1,8-cineole, linalool, 4-
161 terpineol, camphor, (*R*)-borneol, lavandulol, linalyl acetate, (*R*)-(-)-lavandulyl acetate, *n*-decane, *n*-
162 undecane, *n*-dodecane, and *n*-tridecane. ISTD *n*-Pentadecane was included at a concentration of 25 mg/L.

163 Calibration levels investigated were 5, 10, 15, 20, 50, 75, 100, 150, 200, and 250 mg/L for all reference
164 compounds (including linear hydrocarbons *n*-C10 to *n*-C13 adopted for Internal QC).

165 GC-FID purity was controlled for each single component Standard Stock Solution before method validation
166 and results are reported in **Tables 1** and **2** together with Target Ions (*Ti*) adopted for MS quantitation.
167 Enantiomeric composition of chiral markers was determined by ES-GC×2GC-MS/FID and was considered for
168 their calibration.

169 *Mentha* and *Lavandula* spp. EOs samples were prepared at different final concentrations to comply with
170 the method linearity range. Final concentrations were as follows: 5, 2, and 1 mg/mL and 500 µg/mL and
171 were obtained by diluting suitable volumes of a 10 mg/L EO Stock Solution in dichloromethane or
172 cyclohexane. ISTD *n*-Pentadecane at a concentration of 25 mg/L was added to each analyzed sample.

173

174 **GC×GC instrument set-up**

175 GC×GC analyses were run with a system consisting of an Agilent 6890 GC unit provided with a 7683 ALS
176 auto injector sampler (Agilent, Little Falls, DE, USA) coupled to an Agilent 5975C MS detector (Agilent, Little
177 Falls, DE, USA) operating in EI mode at 70 eV. The GC transfer line was set at 280°C. A *Standard Tune* was
178 used and the scan range was set to *m/z* 40-250 with a scanning rate of 12,500 amu/s to obtain a spectra
179 generation frequency of 28 Hz. The Flame Ionization Detector (FID) conditions were: base temperature
180 280°C, H₂ flow 40 mL/min, air flow 240 mL/min, make-up (N₂) 450 mL/min, and sampling frequency 150 Hz.
181 Injections of the EOs and of calibration mixtures, as well as those for I_S^T determination, were by 7683 ALS
182 under the following conditions: split/splitless injector, split mode, split ratio 1/20, injection volume 1µL,
183 and injector temperature 280°C.

184

185 **Thermal modulator parameters**

186 The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation, Houston,
187 TX) cooled with liquid nitrogen controlled by Optimode™ V.2 (SRA Instruments, Cernusco sul Naviglio, MI,
188 Italy). Hot jet pulse time was set at 250 ms; modulation time was 5 s; and cold-jet total flow was

189 progressively reduced with a linear function, from 40% of Mass Flow Controller at initial conditions, to 5%
190 at the end of the run. Loop dimensions were chosen on the basis of the expected carrier linear velocities, to
191 ensure that at least two stage-band-focusing releases were performed for each modulation. Thus, the first
192 0.6 m of the ^2D s was wrapped in the metal slit of the modulator.

193

194 **Column set, connections and auxiliary control module**

195 The column set adopted for *Mentha* spp. quantitative profiling consisted of a primary column of 30 m \times
196 0.25 mm $d_c \times 0.25 \mu\text{m}$ d_f SE52 (95% polydimethylsiloxane, 5% phenyl) connected to two secondary columns
197 of equivalent length of 1.4 m \times 0.1 mm $d_c \times 0.10 \mu\text{m}$ d_f OV1701 (86% polydimethylsiloxane, 7% phenyl, 7%
198 cyanopropyl). The oven temperature was programmed from 50°C (1 min) to 270°C at 3.0°C/min and to
199 290°C at 10°C/min (10 min).

200 For *Lavandula* spp. chiral recognition and quantitative profiling, the column set consisted of a primary
201 column of 25 m \times 0.25 mm $d_c \times 0.25 \mu\text{m}$ d_f of 6^{I-VII}-*O*-TBDMS-2^{I-VII}-3^{I-VII}-*O*-ethyl- β -CycloDextrin as chiral
202 stationary phase (CSP) diluted at 30% in PS086 (DiEt β CD) [22] connected to two secondary columns of
203 equivalent length of 1.4 m \times 0.1 mm $d_c \times 0.10 \mu\text{m}$ d_f OV1701 (86% polydimethylsiloxane, 7% phenyl, 7%
204 cyanopropyl). The oven temperature was programmed from 60°C (1 min) to 180°C at 2.0°C/min and to
205 230°C at 10°C/min (5 min).

206 Connections between the primary and the two secondary columns were by a SilFlow™ GC 3 Port Splitter
207 (SGE Ringwood, Victoria, Australia). The secondary column toward the MS detector was connected to a
208 Quick Swap unit (G3185, Agilent, Little Falls, DE, USA) and to an auxiliary electronic pressure controller
209 (EPC) consisting of a one channel Pneumatics Control Module (G2317A, Agilent, Little Falls, DE, USA). The
210 restrictor capillary in the GC-MS transfer line was of 0.17 m \times 0.1 mm d_c . A schematic picture of the system
211 configuration is provided as a supplementary file (**Supplementary Figure 1 - SF1**). All columns and
212 capillaries were from Mega (Legnano, Milan, Italy). The carrier gas was helium delivered at constant flow
213 with initial head pressure p_i 296.0 KPa and the auxiliary gas for MS outlet pressure correction (He) was
214 delivered at 39.9 KPa (relative). The split ratio (MS/FID) was 50:50. All technical aspects on columns'
215 configuration, connections and auxiliary pressure corrections are discussed in detail in a previous paper by
216 Nicolotti et al. [22].

217

218 **Data acquisition and 2D data automatic processing**

219 Data were acquired by Agilent MSD ChemStation ver D.02.00.275 and processed using GC Image® GC \times GC
220 Edition Software, Release 2.5 (GC Image, LLC Lincoln NE, USA).

221 Calibration curves were automatically generated within GC-Project® by applying a target template that
222 included all calibrated analytes and ISTD. Each target compound in the template was characterized by its ^1D
223 and ^2D retention times, T_i (Quantifier Ion for MS trace only), full mass spectrum (MS trace only), and

224 Qualifier CLIC[®] function to constrain template matching to a minimum MS spectrum similarity value match
225 of 700 (NIST Similarity Match Factor and Reverse Match Factor).

226 Calibration curves, based on external standard responses normalized to ISTD, were generated from single
227 calibration run images after target analyte identifications (template matching) and revision. Each
228 calibration point was defined by 3 replicate runs, so that automatic processing on GC-Project[®] elaborated
229 24 runs (8 levels × 3 replicates) for each application (mint and lavender) and detector channel.

230 A linearity check was done for each detector signal (MS and FID) by arbitrarily fixing a minimum acceptable
231 Coefficient of Determination (R^2) of 0.980. For analytes with $R^2 < 0.980$ calibration was adjusted by
232 excluding external points (see **Tables 1** and **2**) but also including the zero level. Calibration curves for each
233 concentration interval were saved as Calibration Table files and used, in GC-Image[®], for automatic
234 quantitation of EOs.

235 A detailed flow-chart of the automatic processing is available as **Supplementary Figure 2 (SF2)**.

236

237 **Results and discussion**

238 This section reports the validated quantitative results of GC×2GC-MS/FID applied to the analysis of two EOs
239 taken as a model to evaluate this platform. It is divided into three steps: a) validation of the results taking
240 1D-GC-MS as a reference; b) peppermint GC×2GC-MS/FID analysis, in particular dealing with quantitation of
241 minor components eluting close to major peaks; and c) combination of quantitative and enantioselective
242 analysis of lavender EO markers.

243

244 **Method Performance Parameters**

245 Method validation was run over a four-weeks time interval to evaluate the following performance
246 parameters: precision, linearity, and accuracy.

247 Precision data on retention times and on Normalized 2D Peak Volumes (T_i response for MS and total
248 response for FID) were evaluated on three replicate analyses of calibration solutions during the entire
249 validation period. Results on Normalized 2D Peak Volumes are reported in **Table 1** for *Mentha* spp. markers
250 and in **Table 2** for *Lavandula* spp. markers, as average % Relative Standard Deviation (RSD%) values among
251 replicates at each point.

252 Results demonstrate good precision on 2D Volume assessment for both detection channels with the FID
253 showing better repeatability. Exceptions are menthol for the SE52/OV1701 stationary phase combination,
254 which had an average RSD% on replicate analyses on all calibration points of 12.4 (MS) and 10.6 (FID), and
255 (*R*)-(-)-Lavandulol on the DiEtβCD/OV1701 combination, which had an average RSD% of 11.7 for the MS
256 channel. In general, FID gave more repeatable results compared to the MS detection and this trend is
257 confirmed for both column configurations. A possible reason is related to the higher acquisition frequency
258 of FID compared to fast quadrupole MS.

259 Linearity was assessed by linear regression analyses on Normalized 2D Peak Volumes vs. Relative Amount
260 (ISTD *n*-Pentadecane was added at 25 mg/L) within the method working range (5-250 mg/L) and including
261 the zero level. Each calibration curve includes at least six concentration points for each detector (*i.e.*, MS
262 and FID) and column configuration. Zero level was included to facilitate quantitation of minor components
263 (*e.g.*, minor enantiomers), although with a larger relative error.

264 Experimental results on linearity assessment for the SE52/OV1701 stationary phase combination are
265 reported in **Table 1**; for DiEt β CD/OV1701, results are summarized in **Table 2**. Data reported include:
266 calibration ranges; regression line slope, intercept and Coefficients of Determination (R^2); and Average
267 Calibration Error % calculated on residuals at each calibration point.

268 Linearity was generally good with average R^2 of 0.993 and Average Calibration Error % always less than
269 20%. For EO markers quantitation, calibration curves were chosen on the basis of the analytes' expected
270 concentrations and, when possible, confirmed over two different intervals. As for repeatability data, FID
271 detection gave more stable responses resulting in lower quantitation errors. For this reason, the accuracy
272 assessment was performed taking as "reference value" the FID quantitation result.

273 The accuracy was assessed by: (a) checking Internal QC analytes (*n*-alkanes from *n*-C10 to *n*-C13) spiked at a
274 fixed concentration in all samples and (b) by cross-comparison of quantitative results obtained by MS and
275 FID detection (linear regression gave $R^2=0.998$). In all cases, the Absolute Error, calculated as in Equation 1,
276 was less than 20%:

$$277 \text{ (Absolute Error \%)}_i = \left| \frac{[(\text{MS Est. Conc.})_i - (\text{FID Est. Conc.})_i]}{(\text{FID Est. Conc.})_i} \right| * 100 \quad \text{Eq. 1}$$

278 where (MS Est. Conc.)_{*i*} is the experimental concentration estimated within the method linearity range for
279 analyte *i* from the MS signal and (FID Est. Conc.)_{*i*} is from the FID detection channel, arbitrarily considered
280 as the reference value.

281 Accuracy results on *Mentha* and *Lavandula* spp. EOs are reported in **Table 3** and a more detailed and
282 critical discussion on these data follows.

283

284 **Quantitative profiling of *Mentha* spp. essential oils for accurate quality assessment**

285 The QC of *Mentha* spp. EOs focuses on a series of authenticity markers requiring a quantitative profiling
286 approach [26]. Area Percentage (Area %) of limonene, 1,8-cineole, menthone, menthofuran, isomenthone,
287 menthyl acetate, isopulegol, menthol, pulegone, and carvone are assumed as quality markers in the
288 European Pharmacopoeia [21], in the United States Pharmacopeia (USP), and in ISO Reference [27] for
289 peppermint EOs (*Mentha x piperita* L., Lamiaceae). Isopulegol also plays an important role in the
290 authentication and/or adulteration assessment of peppermint with *Mentha arvensis* L. (cornmint) [28,29].
291 Another quality marker of *Mentha spicata* (native spearmint) and *Mentha x gentilis* (scotch spearmint)
292 species, (*R*)-(-)-carvone, is connoted by a distinctive odor note [30,31].

293 One-dimensional GC-FID and GC-MS methods perfectly fit with this purpose (*e.g.*, QC and authentication)
294 and provide more reliable and robust results if based on “true” quantitation of reference markers (based
295 on FID Response Factors - RF or External Standard Calibration - RRFs) instead of relative % abundance
296 profiling [12,13]. However, when EOs are part of a more complex matrix, as in flavorings and/or fragrances,
297 the resulting profile is more complex because of the presence of other components and the responses
298 could be altered by matrix effect phenomena.

299 The validation results on the most informative markers for *Mentha* spp. EOs authentication (**Table 3**),
300 confirm method quantitation reliability. The absolute quantitation error is less than 13% in all cases except
301 for menthol in the *Mentha x gentilis* sample. These data are in agreement with and sometimes better than
302 previous results [12]. A possible reason for more accurate quantitation by GC×GC is its higher separation
303 power that for some analytes results in better chromatographic resolution. This aspect also was noted by
304 Filippi *et al.* [21], who observed significantly different quantitative profiles for vetiver EO components when
305 compared to the ISO reference pattern.

306 The 2D separation patterns of *peppermint* (CS PEPP) and *cornmint* (CS ARV) EOs in the elution region of
307 menthols, shown in **Figures 1B** and **1C**, clearly illustrate how the higher ²D column loadability due to the
308 two-parallel ²D columns, positively affects the overall system performance. For this group of analytes that
309 show similar retention behavior on the ¹D stationary phase (*e.g.*, SE52), extra-chromatographic phenomena
310 such as column overloading, would condition correct separation with detrimental effects on
311 identification/quantitation of minor peaks eluting in the proximity of highly abundant components (*e.g.*,
312 neomenthol and 4-terpineol vs. menthol above all). This critical cluster also is adequately resolved by 1D-
313 GC with high efficiency columns (60 m long × 0.25 mm ID) coated with polar stationary phases (*e.g.*,
314 Carbowax), as those recommended in the official methods [24].

315 Once quantitation consistency on reference analytes and external standard calibration is verified, the next
316 step is the identification of EO constituents by matching MS spectra to those collected in commercial
317 databases and verifying coherence of experimental I_s^T with tabulated ones. **Table 4** reports the list of
318 identified analytes in *Mentha* spp. EO samples together with their retention times in the two
319 chromatographic dimensions (¹D and ²D Rt), experimental and tabulated I_s^T , and mass quantitative
320 descriptors (*i.e.*, Normalized 2D Volumes %).

321 In the successive step, Normalized 2D Volumes (over ISTD *n*-pentadecane at 25 mg/L) from the FID
322 detection channel of identified compounds were adopted for an extended quantitation based on Predicted
323 RRFs.

324 Predicted RRFs were calculated according to the reference formulae [16,17] and normalized to *n*-
325 pentadecane, here adopted as ISTD for normalization.

326 The Relative Response Factor equation is:

$$RRF_i = 10^3 (MW_i/MW_{ISTD})(-61.5 + 88.8n_C + 18.7n_H - 41.3n_O + 3.8n_N, + 64.0n_S - 20.2n_F -23.5n_{Cl} - 10.2n_{Br} - 1.07n_I + 127n_{benz}) \quad \text{Eq. 2}$$

where n_C , n_H , n_O , n_N , n_S , n_F , n_{Cl} , n_{Br} , n_I , and n_{benz} are the number of carbon, hydrogen, oxygen, nitrogen, sulfur, fluorine, chlorine, bromine, and iodine atoms, and the number of benzene rings, respectively. MW_i and MW_{ISTD} are the molecular weights of the analyte i and the ISTD adopted for the development of the model by de Saint Laumer *et al.* [16] (ISTD, methyl octanoate).

The analyte specific RRF was corrected to the *n*-C15/methyl octanoate ratio (*i.e.*, $RRF_{i,n-C15}=0.718/RRF_{i,methyl\ octanoate}$) to adapt the model to *n*-pentadecane.

Results of the extended quantitation of EO components are reported in **Table 4**. Relative quantitation differences indicate good accuracy of the predicted RRF approach for those analytes already quantified by external standard calibration.

Figure 2A shows the distribution of Relative Error % for *Mentha* spp. markers, taking the external standard quantitation approach as reference. Most compounds show good accuracy when quantified using predicted RRFs, although some, such as menthone (CS ARV and CS PEPP) and carvone (CS SPEAR), exceed 20% Relative Error. These data are in good agreement with Tissot *et al.* [17], who compared nominal concentrations of a reference mixture to those estimated by predicted RRF with GC×GC-FID through the Euclidean distances, and with Filippi *et al.* [18], who investigated a high-complexity EO (*i.e.*, Vetiver EO), taking as reference values those estimated by applying Response Factors (RF) specific for the different chemical classes.

In the perspective of quality assessment, experimental results confirm that the *Mentha x piperita* EO (CS PEPP) profile is in agreement with the European Pharmacopeia specifications for both: (a) markers' percentage areas distribution and (b) 1,8-cineole/limonene ratio (reference ratio ≥ 2) [24,28]. Isopulegol content (0.09 %) is in accordance with the authentic peppermint reference pattern [21]. The profiles of the investigated spearmint EOs show some quantitative differences between *Mentha x gentilis* and *Mentha spicata*. In any case, the chemical composition of *Mentha x gentilis* EO is comparable to that reported by Lawrence [33].

Quantitative Profiling and Enantiomeric recognition of *Lavender* spp. essential oils

Once system quantitation reliability with a medium-complexity EO (*e.g.*, *Mentha* spp.) was confirmed, a further dimension of information was included in the analytical platform, *i.e.*, the enantiomeric recognition of chiral markers, in particular, of lavender spp. EOs.

The QC of lavender spp. EOs focuses on a series of authenticity markers requiring a quantitative profiling approach [24]. Area Percentage (Area %) values and/or intervals are reported for linalool, linalyl acetate, lavandulyl acetate, 4-terpineol, lavandulol, 1,8-cineole, camphor, and borneol in the European

361 Pharmacopoeia [24] and in some ISO References [33,34] for *Lavandula angustifolia* Mill. and for *Lavandula*
362 *angustifolia* Mill. x *Lavandula latifolia* Medik. (lavandin Grosso).

363 Another important parameter for quality assessment of lavender EOs is the enantiomeric composition of
364 the chiral components [35]. Among the diagnostic markers, linalyl acetate and linalool are both present in a
365 high enantiomeric excess, greater than 99%, of the (*R*)-(-) form independent of variety, storage, and growth
366 conditions [36].

367 Therefore, the quality assessment of lavender EO should include contemporarily chiral and achiral
368 components quantitative profiling, requiring an analytical platform that combines enantiomeric recognition
369 and high peak capacity suitable to handle the complexity of the resulting 2D pattern. This topic was
370 investigated by Bicchi *et al.* [37,38] for 1D-(ES)-GC-MS, who concluded that a fine tuning of carrier gas
371 linear velocity and temperature rate is mandatory to achieve the resolution of all chiral and achiral markers
372 in a single injection.

373 When a GC×GC platform is adopted, carrier gas linear velocity in the two dimensions cannot be
374 independently optimized and, in many cases, the higher peak capacity compensates for the sub-optimal
375 carrier gas operative conditions. However, to exploit the ¹D chiral recognition by Cyclodextrines (CDs)
376 properly, chromatographic parameters (carrier gas velocity and temperature) should be correctly matched
377 to provide discrimination based on the small difference in the energy of the host/guest interactions
378 between each enantiomer and the chiral selector. The GC×2GC-MS/FID platform operates close-to-optimal
379 in both chromatographic dimensions enabling the full chiral recognition without loss in the overall peak-
380 capacity. **Figure 3** shows the resulting 2D pattern of one *Lavandula angustifolia* Miller EO together with
381 some critical sub-regions in which components coeluting in the ¹D are separated in the ²D.

382 Quantitative results demonstrate fairly good accuracy (**Table 3**) with Absolute Error % values not exceeding
383 18%.

384 When quantitative profiling, based on predicted RRFs, is extended to all analytes identified on the basis of
385 MS spectrum similarity and enantiomer specific I^T_S from a CDs dedicated database [39], accuracy is still
386 acceptable; results are reported in **Table 5**. Relative Errors %, visualized in the histogram of **Figure 2B**,
387 confirm the tendency to overestimate, compared to external standard calibration, already observed for
388 *Mentha* spp. and, with some exceptions, indicate that predicted RRFs results are generally in agreement
389 with those obtained by true quantitation. Interestingly, compounds that exceeded ± 20% error, such as 1,8-
390 cineole and (*S*)-borneol, also exhibited lower accuracy values, although acceptable when considering the
391 absolute concentration in the analyzed samples [40].

392 Experimental results on 2D Volume % confirm that the lavandin EO (CS GROSS) chemical pattern is in
393 agreement with the ISO Reference [34], while within the *Lavandula angustifolia* samples only the CS LAV01
394 shows a profile compatible with the European Pharmacopoeia reference [24]. On the contrary, CS LAV02 and
395 CS LAV03 reports very high percentages of 1,8-cineole, borneol, and camphor, that are outside the range.

396 Enantiomeric distribution of linalool and linalyl acetate, detailed in **Table 6**, leads to exclude the addition of
397 synthetic racemates to the EOs. In particular, the enantiomeric % composition (EC%) of (*R*)-(-)-linalyl
398 acetate is always greater than 97.5%, whereas that of (*R*)-(-)-linalool is generally smaller but still acceptable
399 [24]. (*S*)-(+)-linalool up to 15% may be formed during unusual and extremely time-prolonged
400 hydrodistillation processes [38].

401

402 **Conclusions**

403 The performance of a dual-secondary-column, dual-detection system in an integrated platform for GC×GC
404 has been evaluated and critically discussed in view of its adoption for detailed EO quantitative profiling. In
405 particular, extensive method validation confirmed the platform's reliability in terms of: linearity, precision,
406 and accuracy. This last parameter was assessed by cross-matching quantitative results between the two
407 detectors, *i.e.*, MS and FID. Although the MS detection adds to the system a further analytical dimension
408 that enables unequivocal analytes identification and quantitation, FID response is related to its chemical
409 structure and can be predicted with a reasonable accuracy by mathematical models [16]. When the
410 molecular structure is known, the analyte amount in the sample can be estimated, making it possible to
411 extend the quantitation to all identified compounds of a sample. The experimental data demonstrated the
412 accuracy of the predicted RRFs, supporting their adoption in quantitation of EO markers. This approach is of
413 particular interest in those applications for which reference standards are not (easily) available and/or
414 regulated.

415 Challenging quantitation problems can be overcome thanks to the close-to-optimal ²D linear velocities and
416 doubled loading capacity of the 2nd dimension, as for instance: (a) the accurate quantitation of minor peaks
417 eluting in the proximity of a major component, *e.g.*, neomenthol and 4-terpineol vs. menthol in peppermint
418 and cornmint EOs, and (b) contemporary monitoring and quantitation of chiral and achiral markers without
419 resorting to the MS dimension to resolve co-elutions, *e.g.*, linalool and linalyl acetate in lavender EOs.

420 The GC×2GC-MS/FID platform's quantitation reliability is therefore of high interest, because it matches
421 several analytical needs, enabling an extended quantitative profiling of medium-to-high complexity
422 matrices for both authentication and regulatory purposes. Therefore, its adoption is highly promising in
423 view of its applications to matrices submitted to REACH regulation (Registration Evaluation Authorisation
424 and Restriction of Chemicals) [41], to complex fragrances containing suspected allergens of the EU list, or
425 for upcoming regulations [6,23,43].

426

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429

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498 **Caption to Figures**

499 **Figure 1:** 2D plot of menthol-rich EOs. **Fig. 1A** peppermint (CS PEPP) 2D elution pattern, **Fig. 1B** elution
500 region of menthols in peppermint (*Mentha x piperita* L. - CS PEPP) and **Fig. 1C** in cornmint (*Mentha arvensis*
501 L. - CS ARV).

502

503 **Figure 2:** Relative Error % distribution for *Mentha* spp. (**Fig. 2A**) and lavender (**Fig. 2B**) EOs quality markers
504 quantified through FID RRFs. External Standard quantitation by FID is taken as reference value for accuracy
505 evaluation.

506

507 **Figure 3:** 2D plot of *Lavandula angustifolia* Miller EO (Fig. 3A - CS LAV01) obtained by DiEt β CD/OV1701
508 column combination. Two sub-regions are also shown (Fig. 3B - region I and Fig.3C - Region II) where
509 components are effectively resolved in the ²D.

510

511 **Caption to Tables**

512

513 **Table 1:** Validation results for *Mentha* spp. EOs quality marker quantitation by GC×2GC-MS/FID. Target
514 analytes are reported together with GC-FID purity of reference compounds adopted for external
515 quantitation, Target Ion (*Ti*) adopted for quantitation, calibration interval(s), regression parameters (slope,
516 intercept and Coefficient of Determination R^2), precision (referred as average Relative Standard Deviation %
517 - RSD% between replicates at each calibration point) on Normalized 2D Volume and residual distribution
518 (reported as the Average Calibration Error % on all calibration points) for both detectors.

519

520 **Table 2:** Validation results on quality markers of lavender EOs by GC×2GC-MS/FID with DiEtβCD/OV1701
521 column combination. Target analytes are reported together with GC-FID purity of chiral reference
522 compounds adopted for external quantitation, Enantiomeric Composition %, Target Ion (*Ti*) adopted for
523 quantitation, calibration interval(s), regression parameters (slope, intercept and Coefficient of
524 Determination R^2), precision (referred as Relative Standard Deviation % - RSD%) on Normalized 2D Volume
525 and residual distribution (reported as the Average Calibration Error % on all calibration points) for both
526 detectors.

527

528 **Table 3:** Quantitative results (expressed as g/100 g of EO) obtained by GC×2GC-MS/FID on *Mentha* spp. and
529 lavender EOs under study. Absolute Error % is calculated taken as reference value that obtained by FID.

530

531 **Table 4:** List of identified analytes in *Mentha* spp. EO samples together with their molecular weight (MW)
532 and formula, retention times in the two chromatographic dimensions (1D and 2D Rt), experimental and
533 tabulated I^T_s , mass quantitative descriptors (*i.e.*, Normalized 2D Volumes %), estimated concentration
534 (mg/100g) obtained by predicted FID RRFs and corresponding concentrations (when available) obtained by
535 external standard quantitation.

536

537 **Table 5:** List of identified analytes in lavender EO samples together with their molecular weight (MW) and
538 formula, retention times in the two chromatographic dimensions (1D and 2D Rt), experimental and
539 tabulated I^T_s , mass quantitative descriptors (*i.e.*, Normalized 2D Volumes %), estimated concentration
540 (mg/100g) obtained by predicted FID RRFs and corresponding concentrations (when available) obtained by
541 external standard quantitation.

542

543 **Table 6:** Enantiomeric Composition % of lavender EO samples.

Table 1

Analyte	MS detection channel (Full-SCAN Mode)							FID detection channel					
	GC-FID Purity % (SE52)	Ti (MS Quantifier Ion)	Calibration Interval	Slope	Intercept	R ²	Precision Average Norm. 2D Vol (RSD%)	Average Calibration Error %	Slope	Intercept	R ²	Precision Average Norm. 2D Vol (RSD%)	Average Calibration Error %
Limonene	98.5	93	10-250	0.97	-0.46	0.993	4.4	-10.2	1.17	-0.20	0.992	1.7	-9.3
			0-150	0.96	-0.32	0.994			1.16	-0.14	0.993		
1,8-Cineole	99.8	81	10-250	0.55	-0.11	0.990	6.2	-10.7	1.14	-0.51	0.997	3.6	-1.6
			0-150	0.55	-0.08	0.992			1.14	-0.74	0.989		
Linalool	99.8	71	10-250	0.68	-0.92	0.996	9.4	-5.5	1.18	-1.59	0.995	3.8	-4.5
			0-150	0.66	-0.64	0.993			1.15	-1.10	0.993		
4-Terpineol	96.3	81	10-250	1.15	-0.97	0.998	8.3	-12.0	0.90	-0.20	0.992	4.1	-11.0
			0-150	1.12	-0.68	0.997			0.90	-0.14	0.994		
Menthofurane	94.4	108	10-250	0.16	0.07	0.995	9.6	-6.7	0.88	-0.79	0.996	4.2	-10.7
			0-150	0.17	0.05	0.995			0.87	-0.55	0.995		
Menthol	99	71	10-250	0.94	-0.68	0.996	12.4	-10.5	1.14	-0.57	0.999	10.6	-4.8
			0-150	0.93	-0.47	0.996			1.13	-0.40	0.999		
Menthone	78.1	112	10-250	0.87	-0.77	0.999	6.5	-14.4	1.01	-0.36	0.993	7.6	-16.8
			0-150	0.85	-0.54	0.996			1.00	-0.25	0.994		
Menthyl acetate	92.7	138	10-250	0.38	-0.38	0.998	5.8	-14.4	1.06	0.08	0.992	6.2	-14.5
			0-150	0.37	-0.27	0.995			1.06	0.05	0.994		
Neoisomenthol	54.9	71	10-250	2.33	-0.82	0.994	8.8	-11.4	1.03	0.30	0.969	6.4	-14.2
			0-150	2.29	-0.57	0.994			1.03	0.21	0.968		
Neomenthol	99.8	71	10-250	0.19	-0.23	0.997	9.6	-8.2	1.14	-0.28	0.991	2.7	-6.8
			0-150	0.18	-0.16	0.995			1.14	-0.28	0.991		
Pulegone	98.3	81	10-250	1.04	-1.31	0.999	7.5	-9.3	0.83	-0.19	0.990	7.7	-11.9
			0-150	1.02	-0.91	0.996			0.82	-0.13	0.992		
Carvone	99.8	82	10-250	1.32	-1.62	0.998	8.9	-8.9	0.86	-0.43	0.995	3.6	-9.1
			0-150	1.28	-1.12	0.995			0.85	-0.30	0.996		
Isopulegol	99.5	67	10-250	0.31	-0.07	0.964	7.5	-19.3	0.89	-0.32	0.992	3.0	-9.4
			0-150	0.31	-0.05	0.966			0.88	-0.22	0.993		
Isomenthol	99.8	71	10-250	0.74	-0.71	0.995	8.3	-8.0	0.77	-0.40	0.993	4.9	-5.3
			0-150	0.72	-0.50	0.994			0.76	-0.28	0.994		
Decane	99.8	57	10-250	1.50	0.04	0.986	6.8	-12.5	1.10	-0.25	0.990	3.2	-9.4
			0-150	1.50	0.03	0.988			1.11	-0.17	0.992		
Undecane	99.8	57	10-250	1.45	0.01	0.987	6.1	-11.9	1.00	0.15	0.976	2.1	-12.1
			0-150	1.45	0.01	0.987			1.00	0.10	0.980		
Dodecane	99.7	57	10-250	1.67	0.10	0.985	5.1	-12.4	1.05	-0.14	0.989	2.2	-9.3
			0-150	1.67	0.07	0.988			1.04	-0.10	0.991		
Tridecane	99.3	57	10-250	1.56	-0.02	0.988	6.6	-12.3	1.00	-0.06	0.988	3.3	-9.9
			0-150	1.56	-0.01	0.990			1.00	-0.04	0.991		

Table 2

Analyte	MS detection channel (Full-SCAN Mode)								FID detection channel					
	GC-FID Purity % (SE52)	ES-GC-FID Enantiomeric Composition % (β CD)	T _i (MS Quantifier Ion)	Calibration Interval	Slope	Intercept	R ²	Precision Average Norm. 2D Vol (RSD%)	Average Calibration Error %	Slope	Intercept	R ²	Precision Average Norm. 2D Vol (RSD%)	Average Calibration Error %
(R)-(+)-Limonene	98.5	99 (R)-(+)	93	10-250 0-150	0.98 0.99	-0.50 -0.40	0.999 0.996	4.6	-9.1	1.17 1.18	-0.17 -0.17	0.996 0.995	1.5	-7.9
1,8-Cineole	99.8	-	81	10-250 0-150	0.56 0.52	-0.13 -0.10	0.999 0.996	5.2	-8.7	1.19 1.18	-0.54 -0.75	0.996 0.985	3.5	-3.6
Linalool*	99.8	48 (R)-(-)/52 (S)-(+)	71	10-250 0-150	0.88 0.86	-0.72 -0.64	0.994 0.995	8.6	-4.5	1.18 1.15	-0.88 -0.10	0.996 0.999	3.1	-4.5
4-Terpineol	96.3	81 (S)-(+)/ 19 (R)-(-)	81	10-250 0-150	1.14 1.13	-0.87 -0.77	0.997 0.998	7.2	-10.1	0.94 0.99	-0.30 -0.24	0.997 0.995	4.1	-11.0
(R)-(+)-Borneol	84.7	90 (R)-(+)/10 (S)-(-)	95	10-250 0-150	2.43 2.40	-1.20 -0.83	0.995 0.996	5.7	-13.4	1.03 1.02	-0.50 -0.35	0.999 0.998	2.9	-13.4
Camphor	98.6	51 (R)/ 49 (S)	95	10-250 0-150	1.02 1.01	-0.63 -0.44	0.996 0.996	6.3	-10.1	0.82 0.82	0.05 0.04	0.981 0.985	2.9	-11.9
Lavandulol	82.6	95 (R)/5 (S)	69	10-250 0-150	0.23 0.21	-0.30 -0.14	0.994 0.988	11.7	-17.0	0.90 0.91	-0.01 -0.12	0.982 0.987	9.1	-18.4
linalyl acetate	99	50 (R)-(-)/50 (S)-(+)	93	10-250 0-150	0.93 0.90	-1.15 -0.80	0.999 0.996	9.1	-6.5	0.89 0.87	-0.82 -0.57	0.990 0.990	1.1	-2.1
(R)-(-)Lavandulol acetate	77	99 (R)-(-)	136	10-250 0-150	0.07 0.07	-0.10 -0.07	0.994 0.989	9.8	-16.0	0.98 0.90	-0.78 -0.66	0.998 0.997	5.1	12.7

Table 3

Analyte	<i>Mentha arvensis</i> L. (CS ARV)			<i>Mentha x piperita</i> L. (CS PEPP)			<i>Mentha x gentilis</i> (CS GENT)			<i>Mentha spicata</i> L. (CS SPEAR)		
	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %
	g/100g	g/100g		g/100g	g/100g		g/100g	g/100g		g/100g	g/100g	
Limonene	1.63	1.73	6.1	1.46	1.58	7.5	5.16	5.35	3.5	7.56	7.46	1.3
1,8-Cineole	0.96	1.00	4.3	6.01	5.55	8.2	2.50	2.39	4.7	1.36	1.36	0.2
Linalool	0.14	0.13	11.1	0.34	0.32	7.4	0.06	0.06	7.6	0.19	0.18	8.9
Isopulegol	0.67	0.72	6.4	0.08	0.09	13.0	-	-	-	-	-	-
Menthone ^a	6.44	6.97	7.5	16.68	14.98	11.3	1.22	1.10	10.3	0.18	0.20	5.6
Menthofurane	-	-	-	2.16	2.05	5.3	-	-	-	-	-	-
Neomenthol	1.24	1.23	0.4	2.04	1.92	6.4	-	-	-	-	-	-
Menthol ^b	65.00	69.87	7.0	36.35	38.43	5.4	0.21	0.25	17.0	0.38	0.42	11.3
4-Terpineol	0.40	0.39	0.8	1.01	0.96	4.5	0.14	0.13	5.6	1.06	1.08	1.9
Isomenthol	0.51	0.55	6.0	1.33	1.29	3.8	-	-	-	-	-	-
Neoisomenthol	0.35	0.33	6.4	0.46	0.47	2.0	-	-	-	-	-	-
Pulegone	0.43	0.45	5.7	1.36	1.25	8.3	-	-	-	-	-	-
Carvone ^b	-	-	-	-	-	-	72.20	67.80	6.5	57.66	54.59	5.6
Menthyl acetate	2.55	2.28	11.9	5.62	5.28	6.6	0.06	0.06	4.5	0.07	0.07	2.0
Quality Control Analytes												
Decane	0.15	0.14	5.5	0.15	0.15	2.3	0.16	0.16	1.7	0.16	0.16	6.2
Undecane	0.15	0.15	0.6	0.15	0.15	0.5	0.15	0.15	0.5	0.16	0.16	1.3
Dodecane	0.15	0.16	5.0	0.16	0.15	7.1	0.16	0.16	0.2	0.15	0.16	5.1
Tridecane	0.15	0.16	6.9	0.16	0.16	1.1	0.15	0.16	6.8	0.15	0.15	3.5

Analyte	<i>Lavandula angustifolia</i> (CS LAV01)			<i>Lavandula angustifolia</i> (CS LAV02)			<i>Lavandula angustifolia</i> (CS LAV03)			Lavandin* (CS GROSS)		
	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %	MS(Ti)	FID	Abs. Error %
	g/100g	g/100g		g/100g	g/100g		g/100g	g/100g		g/100g	g/100g	
1,8-Cineole ^a	3.32	3.46	4.0	4.57	4.04	13.2	8.98	10.07	10.8	5.94	7.08	16.0
(S)-(-)-Limonene	0.26	0.26	1.3	0.29	0.28	4.1	1.07	1.02	5.1	0.21	0.20	5.3
(R)-(+)-Limonene	0.72	0.66	9.1	0.90	0.79	13.9	1.19	1.10	8.5	1.27	1.17	8.7
(S)-(-)-Camphor	0.10	0.11	11.6	0.02	0.02	13.8	0.04	0.04	11.4	0.10	0.10	8.3
(R)-(+)-Camphor ^a	2.14	2.08	2.9	4.91	5.40	9.0	7.89	8.48	6.9	5.05	5.10	1.0
(R)-(-)-Linalool ^b	32.34	29.88	8.2	25.80	25.97	0.7	16.91	15.42	9.7	23.90	25.66	6.9
(S)-(+)-Linalool	2.96	2.88	2.8	1.92	1.67	15.0	2.20	1.99	10.3	2.13	2.01	6.0
(2S)-(+)-Borneol	0.30	0.26	15.4	0.07	0.06	17.3	0.15	0.13	14.4	0.07	0.06	9.6
(2R)-(-)-Borneol ^a	1.74	1.62	7.4	1.87	1.98	5.6	5.21	5.84	10.8	2.53	2.21	14.4
(R)-(-)-Linalyl acetate ^b	52.34	56.20	6.9	46.80	41.78	12.0	9.79	10.85	9.7	27.46	32.59	15.8
(S)-(+)-Linalyl acetate	0.88	0.80	10.0	0.26	0.25	7.3	0.27	0.26	3.1	0.88	0.80	10.3
(S)-(+)-4-Terpineol	2.93	2.58	13.7	1.51	1.69	10.4	0.07	0.06	11.5	2.35	2.55	7.9
(R)-(+)-4-Terpineol	0.13	0.12	9.0	0.09	0.09	7.3	0.33	0.30	10.1	0.21	0.18	12.9
(R)-(-)-Lavandulol acetate	1.24	1.18	5.1	2.12	1.80	18.2	1.24	1.14	8.9	2.16	2.07	4.2
(R)-(-)-Lavandulol	0.94	0.92	2.2	0.38	0.36	4.4	0.97	0.84	15.2	0.84	0.80	5.6
Quality Control Analytes												
Decane	0.15	0.14	5.5	0.15	0.15	2.3	0.16	0.16	1.7	0.16	0.16	6.2
Undecane	0.15	0.15	0.6	0.15	0.15	0.5	0.15	0.15	0.5	0.16	0.16	1.3
Dodecane	0.15	0.16	5.0	0.16	0.15	7.1	0.16	0.16	0.2	0.15	0.16	5.1

Tridecane 0.15 0.16 6.9 0.16 0.16 1.1 0.15 0.16 6.8 0.15 0.15 3.5

*: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik

a: quantification was done on EO at 1 mg/L solution

b: quantification was done on EO at 500 µg/L solution

Table 4

Analyte	MW	Formula	<i>Mentha arvensis</i> L. (CS ARV)						<i>Mentha x piperita</i> L. (CS PEPP)			<i>Mentha x gentilis</i> (CS GENT)			<i>Mentha spicata</i> L. (CS SPEAR)			
			¹ D (min)	² D (s)	I _s ^r	I _{s tab} ^s	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal
α-Thujene	136	C10H16	6.92	1.05	938	931	0.03	0.04		0.06	0.05		0.03	0.03		0.02	0.02	
α-Pinene	136	C10H16	7.08	1.09	942	939	0.47	0.71		0.84	0.79		0.31	0.27		0.49	0.45	
Sabinene	136	C10H16	8.58	1.25	978	976	0.18	0.27		0.07	0.08		0.01	0.01		0.30	0.28	
β-Pinene	136	C10H16	8.59	1.26	980	980	0.51	0.77		1.72	1.62		0.77	0.68		0.66	0.60	
Myrcene	136	C10H16	9.17	1.29	992	991	0.09	0.14		0.17	0.16		0.26	0.23		1.35	1.24	
3-Octanol	130	C8H18O	9.42	1.84	998	993	0.20	0.34		0.22	0.24		1.21	1.22		0.91	0.94	
α-Terpinene	136	C10H16	10.17	1.37	1016	1018	-	-		0.21	0.20		-	-		-	-	
p-Cymene	136	C10H16	10.50	1.46	1024	1026	0.02	0.02		0.20	0.17		-	-		-	-	
Limonene	136	C10H16	10.67	1.40	1028	1031	1.18	1.78	1.73	1.72	1.62	1.58	6.27	5.53	5.35	8.42	7.72	7.46
1,8-Cineole	154	C10H18O	10.75	1.62	1030	1033	0.63	1.08	1.00	5.89	6.32	5.55	2.68	2.68	2.39	1.50	1.50	1.36
cis-β-Ocimene	136	C10H16	11.08	1.38	1038	1040	-	-		0.18	0.17		-	-		-	-	
γ-Terpinene	136	C10H16	11.92	1.40	1058	1062	-	-		0.40	0.38		-	-		-	-	
cis-Sabinene hydrate	154	C10H18O	12.25	2.10	1066	1068	-	-		0.89	0.96		0.11	0.11		1.28	1.33	
α-Terpinolene	136	C10H16	13.17	1.45	1088	1088	-	-		0.12	0.11		-	-		-	-	
Linalool	154	C10H18O	13.75	2.07	1102	1100	0.06	0.15	0.13	0.34	0.37	0.32	0.07	0.07	0.06	0.20	0.20	0.18
3-Octyl acetate	172	C10H20O2	14.17	1.72	1111	1124	-	-		0.10	0.12		-	-		0.03	0.04	
Isopulegol	154	C10H18O	15.75	2.11	1145	1146	0.44	0.76	0.72	0.09	0.10	0.09	-	-	-	-	-	-
trans-Sabinol	152	C10H16O	15.75	2.3	1145	1142	-	-		-	-		0.08	0.09		0.05	0.05	
Menthone	154	C10H18O	16.17	2.57	1154	1154	4.93	8.47	6.97	17.03	18.26	14.98	1.30	1.31	1.10	0.19	0.19	0.20
Menthofuran	150	C10H14O	16.50	1.90	1162	1164	-	-	-	1.95	2.18	2.05	0	-	-	-	-	-
Isomenthone	154	C10H18O	16.58	2.47	1164	1164	2.98	5.12		4.86	5.21		0.24	0.24		0.04	0.04	
Neomenthol	156	C10H20O	16.67	2.25	1165	1165	0.88	1.36	1.23	2.02	2.13	1.92	0	-	-	-	-	-
Menthol	156	C10H20O	17.17	2.83	1176	1173	72.32	78.64	69.87	41.12	43.23	38.43	0.27	0.27	0.25	0.45	0.45	0.42
4-Terpineol	154	C10H18O	17.49	2.11	1183	1189	0.24	0.40	0.39	0.96	1.03	0.96	-	0.12	0.13	1.11	1.15	1.08
Isomenthol	156	C10H20O	17.50	2.22	1184	1182	0.27	0.45	0.55	1.07	1.13	1.29	-	-	-	-	-	-
α-Terpineol	154	C10H18O	17.75	2.63	1189	1191	-	-		-	-		0.01	0.42		0.15	0.16	
Neoisomenthol	156	C10H20O	17.75	2.29	1189	1188	0.22	0.37	0.33	0.51	0.53	0.47	-	-	-	-	-	-
Dihydrocarveol	154	C10H18O	18.08	2.47	1196	1192	-	-		-	-		0.93	0.93		1.96	2.03	
trans-Dihydrocarvone	152	C10H16O	18.42	2.45	1204	1200	-	-		-	-		0.12	0.12		0.20	0.21	
trans-Carveol	152	C10H16O	19.25	2.45	1223	1217	-	-		-	-		0.46	0.47		0.26	0.28	

<i>cis</i> -3-Hexenyl isovalerate	184	C11H20O2	19.92	1.85	1238	1240	0.22	0.41											
Pulegone	152	C10H16O	19.92	2.43	1238	1237	0.25	0.44	0.45	1.15	1.26	1.25	-	-	-	-	-	-	-
<i>cis</i> -Carveol	152	C10H16O	19.92	2.44	1238	1242 ^f	-	-					0.55	0.56			0.28	0.30	
Carvone	150	C10H14O	20.42	3.19	1249	1242	-	-	-	0	-	-	72.66	72.82	67.80		67.22	72.68	54.59
Piperitone	152	C10H16O	20.58	2.72	1253	1252	0.26	0.46		0.47	0.51		0.22	0.22			0.14	0.14	
Neomenthyl acetate	198	C12H22O2	21.50	1.98	1273	1275	-	-		0.27	0.32			-				-	
<i>trans</i> -Carvone Oxide	166	C10H14O2	21.75	2.81	1279	1277	-	-					0.16	0.19			0.17	0.21	
Menthyl acetate	198	C12H22O2	22.42	2.11	1294	1294	1.42	2.65	2.28	5.27	6.11	5.28	0.04	0.07	0.06		0.07	0.08	0.07
Isomenthyl acetate	198	C12H22O2	23.17	2.07	1312	1306	-	-		0.02	0.02			-			0.07	0.08	
Dihydrocarvyl acetate	196	C12H20O2	23.92	2.08	1330	1325	-	-					0.54	0.58			0.44	0.49	
<i>cis</i> -Carvyl acetate	194	C12H18O2	25.33	2.1	1364	1362	-	-					0.15	0.17			0.41	0.48	
β -Bourbonene	204	C15H24	26.17	1.70	1384	1384	0.07	0.10		0.43	0.40		0.94	0.81			1.79	1.60	
β -Elemene	204	C15H24	26.50	1.73	1392	1391	0.03	0.04		0.19	0.17		0.19	0.17			0.19	0.17	
<i>trans</i> - β -Caryophyllene	204	C15H24	27.58	1.88	1418	1418	0.19	0.29		2.41	2.24		0.66	0.57			1.20	1.07	
α -Humulene	204	C15H24	29.00	1.84	1452	1454	-	-		0.11	0.10			-				-	
<i>trans</i> - β -Farnesene	204	C15H24	29.25	1.68	1458	1458	-	-		0.31	0.29		0.08	0.07			0.14	0.13	
Germacrene D	204	C15H24	30.17	1.93	1480	1480	0.07	0.11		2.12	1.96			-				-	
Bicyclogermacrene	204	C15H24	30.75	1.87	1494	1494	-	-		0.34	0.32			-				-	
δ -Cadinene	204	C15H24	31.83	1.73	1520	1524	0.02	0.04		0.08	0.07			-				-	
Spathulenol	220	C15H24O	33.92	2.42	1570	1576	0.02	0.04		0.05	0.05			-				-	
Caryophyllene oxide	220	C15H24O	34.08	2.44	1574	1581	0.04	0.06		0.12	0.12		0.50	0.48			0.19	0.19	
Viridiflorol	222	C15H26O	34.42	2.35	1582	1590	-	-		0.45	0.45			-				-	

S: R.P. Adams Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition Allured Publishing Corporation, 2007, Carol Stream US
 £: Hognadottir, A., Rouseff, R.L. *J. Chromatogr. A.* **2003**, 998, 201

Table 5

Analyte	MW	Formula	<i>Lavandula angustifolia</i> (CS LAV01)						<i>Lavandula angustifolia</i> (CS LAV02)			<i>Lavandula angustifolia</i> (CS LAV03)			Lavandin* (CS GROSS)			
			¹ D (min)	² D (s)	<i>I</i> _s ^T	<i>I</i> _{s tab} ^S	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal	2D Volume %	g/100 g PRF	g/100 g Ext Cal
α-Pinene	136	C10H16	8.42	1.07	930	924	0.17	0.19		0.42	0.42		0.75	0.80		0.44	0.49	
(1 <i>S</i> ,4 <i>R</i>)-(-)-Camphene	136	C10H16	8.42	1.17	930	917	0.09	0.09		0.04	0.04		0.08	0.08		0.04	0.04	
(1 <i>R</i> ,4 <i>S</i>)-(+)-Camphene	136	C10H16	9.00	1.14	945	934	0.07	0.08		0.28	0.28		0.47	0.50		0.22	0.25	
(1 <i>R</i>)-(+)-β-Pinene	136	C10H16	9.58	1.25	960	947	0.07	0.07		0.18	0.18		0.31	0.34		0.28	0.30	
(1 <i>S</i>)-(-)-β-Pinene	136	C10H16	10.00	1.24	968	957	0.07	0.07		0.15	0.14		0.27	0.29		0.20	0.22	
(1 <i>R</i> ,5 <i>R</i>)-(+)-Sabinene	136	C10H16	10.75	1.20	982	973	0.03	0.04		0.08	0.08		0.16	0.17		0.12	0.13	
1,8-Cineole	154	C10H18O	11.08	1.72	989		2.65	3.28	2.70	4.00	4.50	3.59	9.16	11.12	8.39	6.14	7.69	5.90
Myrcene	136	C10H16	11.58	1.22	998		0.51	0.55		0.78	0.77		0.92	0.88		0.58	0.64	
δ-3-Carene	136	C10H16	13.25	1.20	1030		0.12	0.13		0.06	0.06		0.06	0.07		0.08	0.09	
1-Hexanol	102	C6H14O	13.75	0.92	1040	1042	0.09	0.13		0.07	0.09		0.13	0.20		0.08	0.12	
(<i>E</i>)-3-Hexen-1-ol	100	C6H12O	14.50	0.88	1054		0.07	0.10		-	-		1.92	2.59		0.04	0.06	
(-)-β-Phellandrene	136	C10H16	14.50	1.29	1054		0.13	0.14		0.21	0.20		0.04	0.04		0.02	0.02	
(<i>S</i>)-(-)-Limonene	136	C10H16	14.83	1.21	1060	1057	0.25	0.27	0.26	0.29	0.29	0.28	1.01	1.08	1.02	0.19	0.21	0.20
trans-β-Ocimene	136	C10H16	15.42	1.31	1071		1.75	1.91		1.01	1.00		0.50	2.17		0.32	0.35	
<i>cis</i> -β-Ocimene	136	C10H16	15.50	1.23	1073	1071	1.92	2.09		0.78	0.77		2.04	0.53		1.05	1.16	
(<i>R</i>)-(+)-Limonene	136	C10H16	15.58	1.17	1075	1072	0.51	0.56	0.53	0.99	0.98	0.93	1.19	1.27	1.20	0.94	1.04	0.98
(+)- <i>trans</i> Linalool oxide	170	C10H18O2	16.75	1.68	1097	1094	0.17	0.25		0.07	0.09		0.09	0.12		0.09	0.14	
(-)- <i>trans</i> Linalool oxide	170	C10H18O2	17.10	1.68	1101	1101	0.04	0.05		0.03	0.04		0.02	0.02		0.04	0.05	
(-)- <i>cis</i> Linalool oxide	170	C10H18O2	17.17	1.83	1105	1101	0.15	0.22		0.04	0.05		0.07	0.10		0.07	0.10	
<i>trans</i> -Sabinene hydrate	154	C10H18O	17.42	1.66	1109		0.04	0.05		0.05	0.05		0.05	0.12		0.10	0.12	
Octen-1-ol acetate	170	C10H18O2	17.42	1.80	1109	1105	0.43	0.61		0.30	0.39		0.10	0.06		0.27	0.38	
1-Octen-3-ol	128	C8H16O	18.25	1.10	1125		0.24	0.31		0.08	0.09		0.47	0.58		0.13	0.17	
(<i>S</i>)-(-)-Camphor	152	C10H16O	19.25	2.37	1143	1136	0.08	0.10	0.09	0.01	0.02	0.02	0.02	0.03	0.03	0.06	0.08	0.08
Hexyl propanoate	158	C9H18O	19.33	1.57	1145		0.19	0.26		0.12	0.15		0.20	0.27		0.12	0.17	
(<i>R</i>)-(-)-Camphor	152	C10H16O	19.58	2.41	1149	1142	1.55	1.95	1.91	5.60	6.43	6.33	8.36	10.35	10.21	4.49	5.73	5.63
(<i>R</i>)-(-)-Linalool	154	C10H18O	20.75	1.76	1171	1169	27.93	34.58	24.90	27.13	30.52	22.05	33.33	17.96	13.26	24.06	30.10	21.76
(<i>S</i>)-(+)-Linalool	154	C10H18O	21.83	1.52	1191		1.87	2.32	2.15	1.45	1.63	1.65	1.51	1.83	1.79	1.57	1.97	1.89
(2 <i>S</i>)-(+)-Borneol	154	C10H18O	22.50	2.18	1203	1195	0.23	0.28	0.23	0.05	0.06	0.05	0.13	0.16	0.13	0.07	0.09	0.07
(2 <i>R</i>)-(-)-Borneol	154	C10H18O	22.83	2.20	1209	1202	1.11	1.38	1.29	2.02	2.27	2.01	6.19	7.52	6.26	1.96	2.45	2.16
Hexyl butanoate	172	C10H20O2	23.50	1.77	1220	1215	0.28	0.39		0.58	0.73		0.37	0.51		0.33	0.47	
(<i>R</i>)-(-)-Linalyl acetate	196	C12H20O2	24.58	2.66	1239	1232	32.01	43.64	40.50	15.80	37.18	34.59	7.60	10.16	9.85	26.24	36.15	33.64
(<i>S</i>)-(+)-Linalyl acetate	196	C12H20O2	24.92	2.24	1245	1240	0.61	0.83	0.71	0.24	0.29	0.25	0.06	0.29	0.25	0.39	0.54	0.46
(<i>S</i>)-(+)-4-Terpineol	154	C10H18O	25.17	1.74	1249	1247	1.80	2.23	2.12	1.56	1.76	1.70	2.59	0.07	0.06	2.39	2.99	2.79
Hexyl-2-methyl butyrate	186	C11H22O2	25.17	1.92	1249		0.06	0.08		0.13	0.16		0.22	3.43		0.08	0.11	
(<i>R</i>)-(-)-4-Terpineol	154	C10H18O	25.50	1.64	1255	1253	0.09	0.11	0.10	0.08	0.09	0.08	0.21	0.26	0.22	0.13	0.17	0.15
Hexyl Isovalerate	184	C11H20O2	25.58	1.90	1256		0.06	0.08		0.13	0.16		0.10	0.13		0.17	0.24	
(<i>R</i>)-(-)-Lavandulyl acetate	196	C12H20O2	26.33	2.52	1269	1263	0.82	1.11	0.93	1.77	2.20	2.08	0.82	1.10	0.91	2.15	2.97	2.67
(<i>R</i>)-(-)-Lavandulol	154	C10H18O	26.58	1.57	1274	1275	0.73	0.90	0.82	0.30	0.34	0.31	0.56	0.68	0.62	0.54	0.68	0.62
(<i>S</i>)-(-)-α-Terpineol	154	C10H18O	27.92	1.63	1297	1297	0.29	0.36		0.19	0.21		0.25	0.31		0.27	0.34	
(<i>R</i>)-(+)-α-Terpineol	154	C10H18O	28.58	1.58	1309	1310	0.81	1.00		0.56	0.64		0.71	0.87		0.73	0.91	
Hexyl Tiglate	184	C11H20O2	31.42	2.05	1362	1359	0.07	0.09		0.22	0.27		0.08	0.11		0.15	0.21	
Geraniol	154	C10H18O	31.67	1.74	1367	1368	0.51	0.63		0.29	0.32		0.30	0.36		0.35	0.44	
Neryl Acetate	196	C12H20O2	32.17	2.31	1376	1372	0.42	0.57		0.24	0.30		0.18	0.24		0.25	0.34	
α-Santalene	204	C15H24	33.33	2.16	1398	1391	0.43	0.46		0.11	0.10		0.20	0.21		0.21	0.22	
(-)- <i>trans</i> Caryophyllene	204	C15H24	34.00	2.28	1410	1405	1.81	1.94		1.98	1.93		1.39	0.95		1.53	1.66	

Geranyl acetate	196	C ₁₂ H ₂₀ O ₂	34.17	2.20	1413	1411	1.29	1.75	0.02	0.03	0.04	0.65	0.55	0.75
<i>trans</i> -β-Farnesene	204	C ₁₅ H ₂₄	36.50	2.13	1457	1454	0.88	0.94	0.94	0.91	1.02	1.07	1.17	1.27
Germacrene D	204	C ₁₅ H ₂₄	37.17	2.42	1469	1464	0.17	0.18	0.47	0.45	0.41	0.43	0.58	0.62
Lavandulyl isovalerate	238	C ₁₅ H ₂₆ O ₂	38.42	2.63	1492	1489	0.08	0.11	0.23	0.27	0.31	0.39	0.26	0.34
β-Bisabolene	204	C ₁₅ H ₂₄	39.42	2.13	1511	1509	0.06	0.07	0.09	0.09	0.07	0.07	0.12	0.13
α-Bisabolol	222	C ₁₅ H ₂₆ O	50.08	2.67	1719	1724	0.06	0.08	0.56	0.59	0.31	0.35	0.27	0.32

§: E. Liberto, C. Cagliero, B. Sgorbini, C. Bicchi, D. Sciarrone, B.D. Zellner, L. Mondello, P. Rubiolo, *J. Chromatogr. A.* **2008**, 1195, 117.

Table 6

Analyte	Enantiomeric Composition % (EC%)			
	CS LAV01	CS LAV02	CS LAV03	CS GROSS
(R)-(-)-Linalool	85.6	94.9	90.7	93.9
(S)-(+)-Linalool	14.4	5.1	9.3	6.1
(R)-(-)-Linalyl acetate	98.1	99.2	97.2	98.6
(S)-(+)-Linalyl acetate	1.9	0.8	2.8	1.45

Figure 1

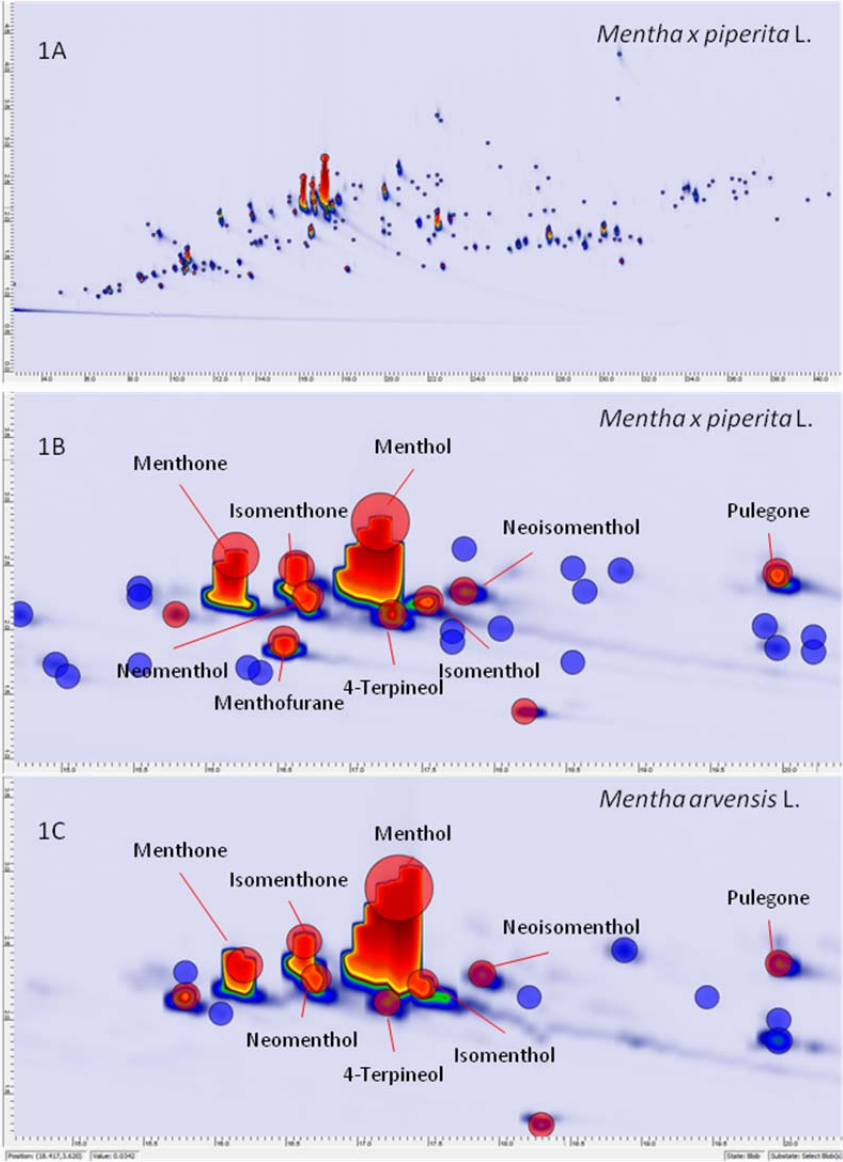
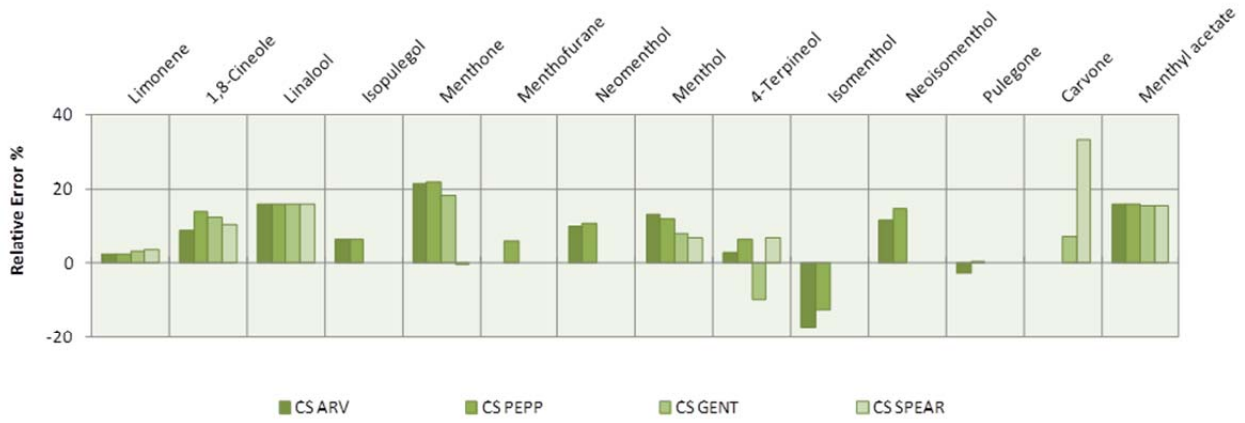


Figure 2

2A



2B

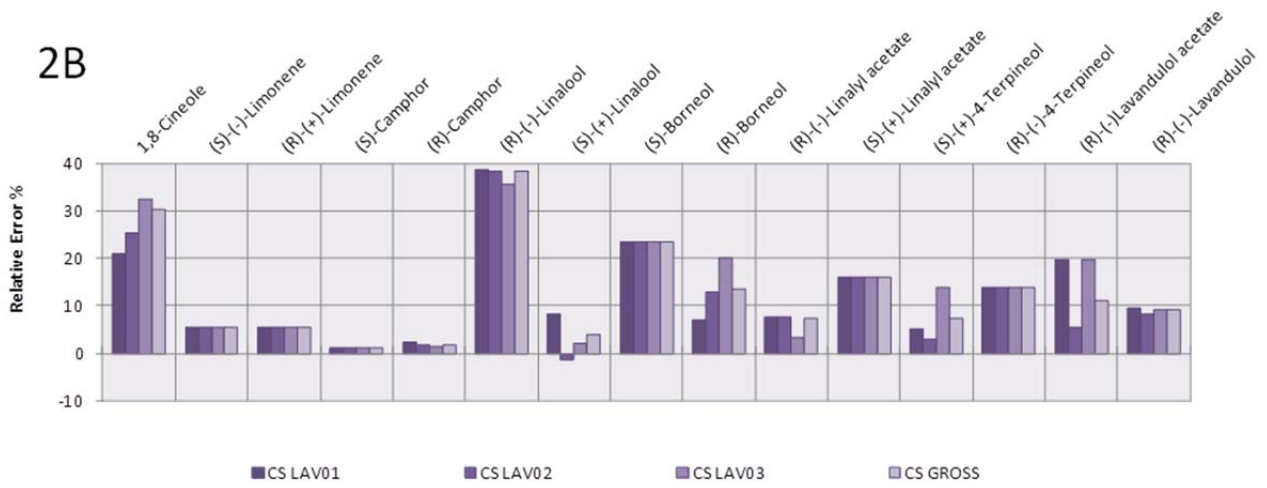
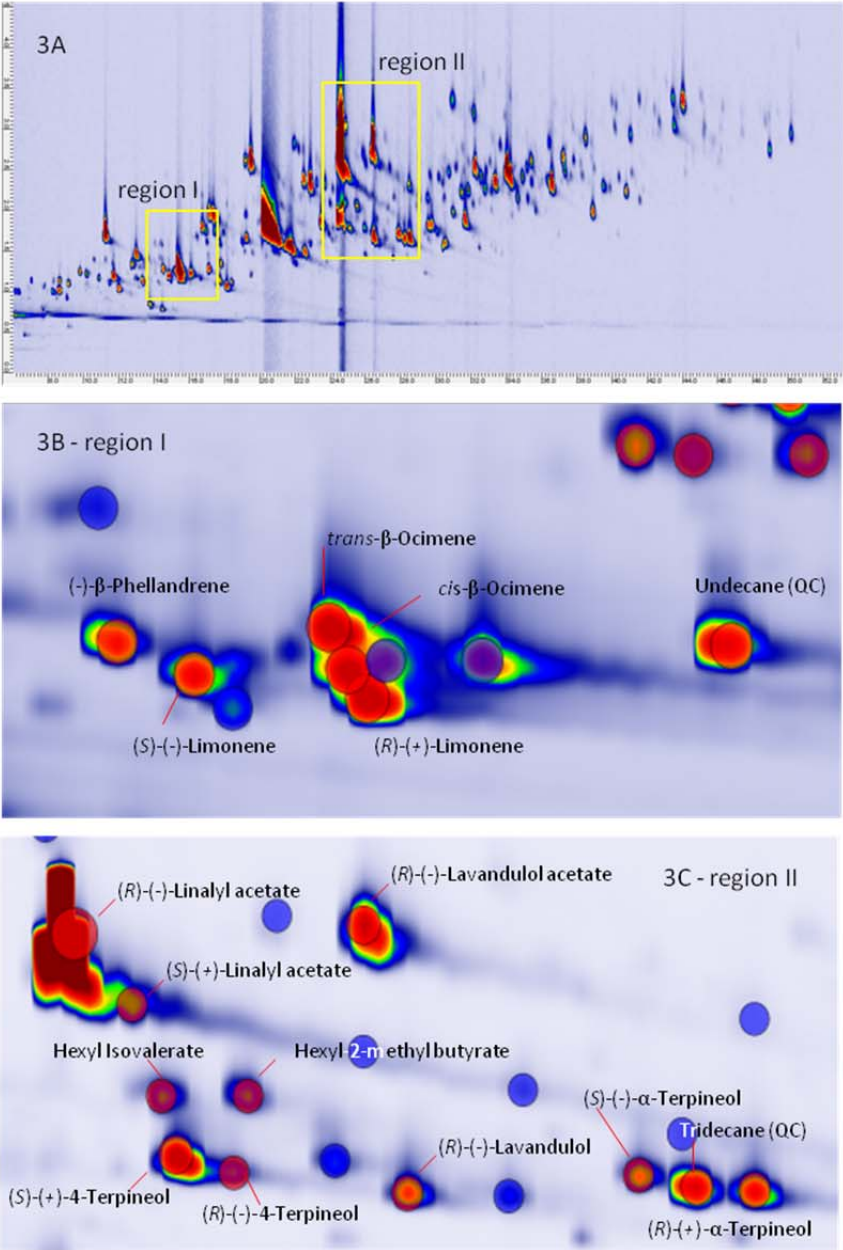
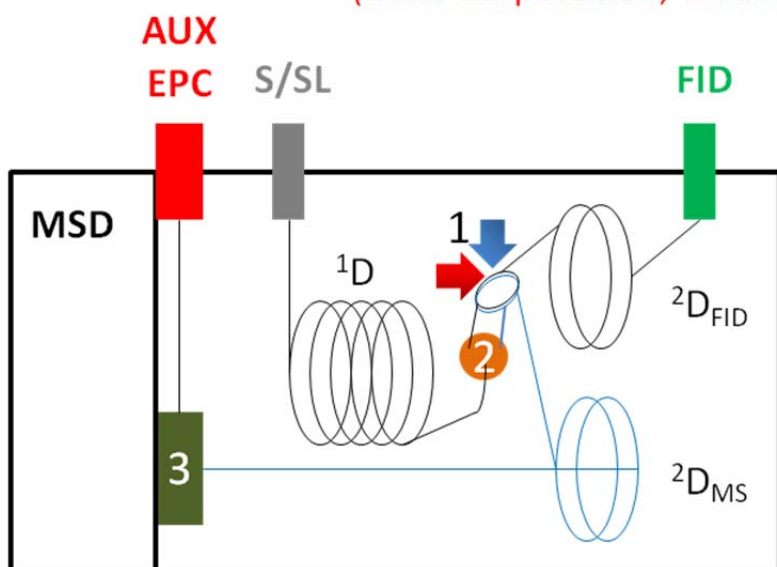


Figure 3



Supplementary Figure 1

1. Loop-Type thermal modulator
(Zoex Corporation, Houston, TX)



2. Microfluidic 3-port splitter
(Sil-flow™- SGE Ringwood,
Victoria, Australia)

3. Outlet pressure compensation
Microfluidic device (Quick-Swap™- Agilent)