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Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils

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# Food Control

## Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils

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<b>Abstract:</b>	<p>The requirement for developing an instrumental method for analysis of volatile compounds responsible for the aroma that supports the work of the sensory panel test of virgin olive oils is a matter of great importance. In this paper, five laboratories participated in a collaborative study within the EU H2020 OLEUM project to develop a peer interlaboratory study of a harmonized SPME-GC-MS method for determination of volatile compounds in virgin olive oil responsible for positive attributes (e.g. fruity) and the main sensory defects. Linearity (<math>R^2 &gt; 0.94</math>) and repeatability (mean relative standard deviation, <math>RSD\% = 7.60\%</math>) were satisfactory. Reproducibility results were uneven depending on the compound. The lowest <math>RSD\%</math> values were found for (Z)-3-hexenyl acetate (19.19%), 1-hexanol (13.26%), and acetic acid (17.47%). The limits of quantification were <math>&lt; 0.07</math> mg/kg for all compounds except for (E)-2-decenal and pentanoic acid. The study of different quantification methods revealed that the correction of the calibration curves using the internal standard led to a slightly worse repeatability, but better accuracy and reproducibility. The results obtained by five laboratories are preparatory towards a trial proper validation study, already planned in OLEUM project, involving external labs participating on a voluntary basis.</p>
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1 **Collaborative peer validation of a harmonized SPME-GC-MS method**

2 **for analysis of selected volatile compounds in virgin olive oils**

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23 **Abstract**

24           The requirement for developing an instrumental method for analysis of volatile compounds  
25 responsible for the aroma that supports the work of the sensory panel test of virgin olive oils is a  
26 matter of great importance. In this paper, five laboratories participated in a collaborative study within  
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28 MS method for determination of volatile compounds in virgin olive oil responsible for positive  
29 attributes (e.g. fruity) and the main sensory defects. Linearity ( $R^2 > 0.94$ ) and repeatability (mean  
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31 depending on the compound. The lowest  $RSD\%$  values were found for (*Z*)-3-hexenyl acetate  
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33 mg/kg for all compounds except for (*E*)-2-decenal and pentanoic acid. The study of different  
34 quantification methods revealed that the correction of the calibration curves using the internal  
35 standard led to a slightly worse repeatability, but better accuracy and reproducibility. The results  
36 obtained by five laboratories are preparatory towards a trial proper validation study, already planned  
37 in OLEUM project, involving external labs participating on a voluntary basis.

38 **Keywords:** virgin olive oil; volatile compounds; sensory analysis; SPME-GC-MS; collaborative trial  
39 validation.

40 **Declaration of competing interest:** None.

41

## 42 **1. Introduction**

43           The analysis of volatile organic compounds (VOCs) has been identified as the best approach  
44 for supporting the current official method of sensory evaluation of positive and negative attributes  
45 (García-González et al., 2011; Morales et al., 2013; Cecchi et al., 2019; Valli et al., 2020; Aparicio-  
46 Ruiz et al., 2019; Valli et al., 2020). Today, the evaluation of these attributes (presence/absence and  
47 their intensity) is carried out according to a method known as panel test (IOC, 1987 and subsequent  
48 amendments) which is the official method to classify virgin olive oils (VOOs) according to their  
49 aroma and organoleptic characteristics (EEC, 1991 and subsequent amendments). In fact, VOO is the  
50 only edible oil product with international regulations requiring official sensory analysis carried out  
51 by panelists to verify commercial categories (García-Vico et al., 2017; García-González et al., 2018).  
52 However, the panel test is subject to some weaknesses and limitations (García-González & Aparicio,  
53 2004; García-González et al., 2007; Aparicio-Ruiz et al., 2019). Thus, debated classifications are  
54 sometimes observed as well as misalignments in the classification carried out by different panels  
55 (Barbieri et al., 2020a). These problems have promoted the investigation of instrumental tools to  
56 support the daily work of panelists and to overcome other known drawbacks, such as the length and  
57 cost of the sensory analysis procedure and the limited number of panels (Aparicio-Ruiz et al., 2019;  
58 Romero et al., 2015; Casadei et al., 2021) in addition to the recommendations for managing a panel  
59 in emergency circumstances, such as a pandemic (IOC, 2020). To mitigate these drawbacks, an  
60 instrumental method based on the analysis of VOCs is required with the objective of providing  
61 additional analytical information to reinforce VOO classification into quality categories. These  
62 methods can be based on untargeted approaches with the aid of chemometric classification (García-  
63 González & Aparicio, 2004; Quintanilla-Casas et al., 2020; Garrido-Delgado et al. 2011, Valli et al.,  
64 2020; Barbieri et al., 2020b) or targeted determination of individual volatile markers as they are key  
65 odorants of VOO aroma (Aparicio et al., 2012; Morales et al., 2013; Servili et al., 2015; Cecchi et al.,  
66 2019; Casadei et al., 2021). In the targeted determination, prior to proposing a classification scheme

67 based on concentration ranges or decision rules, it is necessary to evaluate the performance of the  
68 method in quantitative terms with an interlaboratory perspective. Thus, in addition to intra-lab  
69 validation studies ([Romero et al., 2015](#); [Aparicio-Ruiz et al., 2018](#); [Cecchi et al., 2019](#)), the aim is to  
70 propose a daily routine method that is focused on detection of a minimum number of selected  
71 diagnostic markers. Moreover, an inter-lab study was also carried out to check the results when  
72 slightly different conditions are applied (e.g. different column brands, different GC instrument and  
73 equipment).

74 Although several analytical solutions have been proposed for VOO quality control, to date the  
75 regulatory bodies are unwilling to adopt them, partially due to the lack of a harmonized protocol that  
76 is accepted and internationally applied and the lack of inter-lab performance evaluation. One of the  
77 main sources of variability in the methods is the extraction technique to concentrate volatile  
78 compounds ([Morales et al., 2013](#)). In the last years, methods based on SPME are gaining importance  
79 in relation to other approaches because of their simplicity and efficiency in extraction, not only in  
80 VOO analysis ([Vichi et al., 2003](#); [Morales 2013](#)), but also in the quality control of other foods  
81 ([Giuffrida et al., 2005](#); [Jimenez-Alvarez et al., 2008a, 2008b](#)). [Kanavouras et al.](#) ([Kanavouras,](#)  
82 [Kiritsakis & Hernandez, 2005](#); [Kanavouras & Hernandez, 2006](#)) compared the isolation capability  
83 between Tenax trapping and HS-SPME. They observed that a larger amount of volatile compounds  
84 was isolated when applying the first technique, while the second was quicker and led to a more rapid  
85 descriptive analysis of oxidized VOOs. On the other hand, [Servili et al. \(2004\)](#) compared the Head-  
86 Space Analysis (HSA) of volatile compounds in olive oils using SPME-GC/MS, electronic nose and  
87 Proton Transfer Reaction (PTR)-MS in terms of their capacity to classify VOOs according to the  
88 variety, geographical origin and ripening stage of the fruit.

89 Another source of variability in the analytical methods is the detector. In this regard, recently,  
90 another comparative study was carried out on two SPME-GC methods: SPME-GC-mass  
91 spectrometry (MS) and SPME-GC-Flame Ionization Detector (FID) ([Aparicio-Ruiz et al., 2018](#)). The

92 results and the experience working with both detectors highlighted that the two options provide  
93 advantages, and thus it is necessary to evaluate the performance of methods based on the two  
94 detectors. FID is a robust and low-cost option, and commonly used in all the labs working on quality  
95 control of VOO. On the other hand, MS facilitates the identification of volatile compounds, which is  
96 particularly advantageous in VOO aroma given the presence of a large number of volatile compounds  
97 (Morales et al., 2013; Cecchi et al., 2021). With the aim of developing analytical instrumental  
98 methods to support the panel test, the European Union has encouraged the development,  
99 harmonization and validation of such methods through the Horizon 2020 funded project OLEUM  
100 (Casadei et al., 2021). Within the frame of this project, a harmonized method with two possible  
101 detectors has been developed (SPME-GC-FID and SPME-GC-MS) to analyze volatile compounds in  
102 VOOs. The harmonization includes the definition and set up of all the possible variables that were  
103 identified as sources of errors, such as GC column, SPME fiber composition and length, vial volume,  
104 and internal standard, as well as the calibration and quantification procedures (Casadei et al., 2021).  
105 The performance of the method based on SPME-GC-FID has been evaluated in a peer interlaboratory  
106 study by three different laboratories involved in the OLEUM project (Casadei et al., 2021). With the  
107 same objective, in the present work, five laboratories, all being active partners in the OLEUM project,  
108 carried out an inter-lab evaluation of the SPME-GC-MS joint protocol. The validation was carried  
109 out by each laboratory following the same analytical conditions and on the same samples, in order to  
110 make the results obtained by each laboratory comparable in a harmonized procedure and  
111 methodology, as previously done with FID (Casadei et al., 2021). Aside from the detector, the  
112 analytical variables are the same as those used in SPME-GC-FID, as well as the analyzed samples  
113 and the time frame given to the labs to provide their data. For these reasons, the outcomes of this  
114 work are also comparable with the results obtained by Casadei et al. (Casadei et al., 2021). Although  
115 the primary objective of this investigation is not to compare the results from SPME-GC-FID and  
116 SPME-GC-MS, some conclusions comparing the analytical parameters will be provided.



## 117 2. Materials and Methods

### 118 2.1. Chemicals

119 [Table 1](#) shows the VOCs studied in this work. The pure standards of these compounds were  
120 purchased from Merck KGaA (Darmstadt, Germany). The CAS number and purity of each of the  
121 standards are also shown in [Table 1](#). Additionally, a mixture of *n*-alkanes from 8 to 20 carbon atoms  
122 (~ 40 mg/L each, in *n*-hexane) and 4-methyl-2-butanol (purity  $\geq 98\%$ ) were also purchased from the  
123 same supplier for calculation of the linear retention indexes (LRI) and its use as internal standard (IS),  
124 respectively.

### 125 2.2. Samples

126 For this study, a set of 15 samples were selected for the peer inter-laboratory validation study  
127 of the SPME-GC-MS method. The selection was carried out to possibly cover the natural ranges of  
128 concentration normally present in VOOs and were the same samples used in a previous study on  
129 SPME-GC-FID performance ([Casadei et al., 2021](#)). These samples were sensory evaluated in the  
130 course of the OLEUM project by six panels ([Barbieri et al., 2020a](#)) to have accurate information on  
131 their commercial categories. Thus, these samples were categorized as 3 extra virgin (EV), 6 virgin  
132 (V), and 6 lampante (L) olive oils. In Vs and Ls, 6 oils were graded as rancid, 3 as fusty/muddy  
133 sediment, 2 as musty-humid-earthly and 1 as winey-vinegary according to the main perceived defect  
134 reported by the panelists.

### 135 2.3. Internal standard (IS) solution and sample preparation

136 The IS solution was prepared as described by [Casadei et al. \(2021\)](#). For this purpose, 4-methyl-  
137 2-pentanol, the IS used in this work, was diluted in refined oil to have an approximate concentration  
138 of 50 mg/kg. The weights during this preparation were used to calculate the exact concentration. The  
139 sample was also prepared following the procedure by [Casadei et al. \(2021\)](#) in which 0.1 g of the IS

140 solution was added to 1.9 g of the VOO sample to have an approximate concentration of 2.5 mg/kg.  
141 The exact concentration was also calculated by considering the weights in the preparation.

#### 142 *2.4. Gas chromatographic coupled to mass spectrometer analysis*

143 The sample, placed in a 20 mL vial closed with a septum (polytetrafluoroethylene), was left  
144 for 10 min at 40 °C under agitation to allow for equilibration of the volatiles in the headspace. After  
145 that, the SPME fiber was exposed to the headspace for 40 min at 40 °C. The fiber was then inserted  
146 into the injector port of the GC. [Table 2](#) describes the specific characteristics of the analysis carried  
147 out by the five labs that applied the joint protocol: University of Udine, University of Perugia, ITERG,  
148 University of Barcelona, and Nestlé Research Center, coded as Laboratory 1-5 respectively. The  
149 volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at  
150 250 °C with the purge valve off (splitless mode) and injected into a capillary column of a gas  
151 chromatograph with a mass spectrometry detector. The capillary column was of a polar phase based  
152 on polyethylene glycol (PEG) (e.g. ZB-WAX or TR-WAX), length 60 m, internal diameter 0.25 mm  
153 and coating 0.25 – 0.50 µm. The specific column brand and characteristics of each lab are shown in  
154 [Table 2](#). The transfer line temperature was set at 260 °C. The temperature of the ion source was set  
155 according to the technical specifications of each instrument. The carrier gas used by the five labs was  
156 helium, although this parameter was not specified in the harmonized protocol. The oven temperature  
157 was held at 40 °C for 10 min and then programmed to increase by 3 °C/min to a final temperature of  
158 200 °C. A cleaning step was added at the end of the oven programmed temperature by all participants  
159 (20 °C/min to 250 °C for 5 min) to ensure that the column was ready for the next analysis.

#### 160 *2.6. Identification and quantification of VOCs*

161 Linear Retention Index (LRI) and standards were used for identification ([Casadei et al., 2021](#))  
162 in addition to mass spectrometry (MS databases of each lab shown in [Table 2](#)). [Table 1](#) shows the  
163 characteristic m/z of each compound to be used in the integration with the extracted ion

164 chromatogram mode. The positive ionization mode was used in the 5 labs. [Figure 1](#) shows the  
165 chromatogram of L and V samples.

166 The quantification of selected VOCs was carried out by the three quantification methods  
167 described by [Casadei et al. \(2021\)](#), named QM1, QM2, and QM3. These three methods were applied  
168 by the five labs using the same Excel files for the calculations. QM1 and QM2 used the calibration  
169 curves with the equations  $A_{\text{Analyte}}/A_{\text{IS}} = m_{\text{QM1}} \cdot C_{\text{Analyte}}$  and  $A_{\text{Analyte}} = m_{\text{QM2}} \cdot C_{\text{Analyte}}$ , respectively; where  
170  $A_{\text{Analyte}}$  is the area corresponding to the analyte,  $A_{\text{IS}}$  is the area corresponding to the IS used in building  
171 the calibration curves and  $m_{\text{QM1}}$  is the slope of the calibration curve. QM3 was based in the equation  
172  $(A_{\text{Analyte}} / A_{\text{IS}}) = (m_{\text{Analyte}}/m_{\text{IS}}) \cdot (C_{\text{Analyte}} / C_{\text{IS}})$ ; where  $A_{\text{Analyte}}$  is the area corresponding to the analyte,  
173  $A_{\text{IS}}$  is the area corresponding to the IS,  $m_{\text{IS}}$  is the slope of the calibration curve built for IS,  $m_{\text{Analyte}}$  is  
174 the slope of the calibration curve built for the analyte,  $C_{\text{Analyte}}$  is the concentration corresponding to  
175 the analyte, and  $C_{\text{IS}}$  is the concentration of the IS in the sample ([Kalua, Bedgood, & Prenzler, 2006](#)).

## 176 *2.7 Calibration curves*

177 The quantification of the selected VOCs in the headspace of VOOs was carried out by using  
178 calibration curves that were built as linear regression (intercept equal to 0), for the 18 VOCs described  
179 in [Table 1](#). These calibration curves were prepared using standard mixtures (SMs), as reported in  
180 [Casadei et al., 2021 \(Casadei et al., 2021\)](#), instead of preparing dilutions for each single compound.  
181 The two mixtures, coded as SM-A and SM-B ([Table 1](#)), were prepared to have a concentration of  
182 10,000 mg/kg for each VOCs, and were used to have subsequent dilutions, coded as SM1 (200  
183 mg/kg), SM2 (20 mg/kg) and SM3 (2 mg/kg). SM1 was prepared by adding 5 g of refined olive oil  
184 in a 20 mL vial. Next, 0.2 g of SM-A or SM-B was added and more refined olive oil was added to  
185 reach a total of 10 g. In order to prepare SM2, 1 g of SM1 was added to 5 g of refined olive oil. SM3  
186 was likewise prepared by adding 1 g of SM2 to 5 g refined olive oil. The necessary weights of refined  
187 oil and these three standard mixtures to obtain these concentrations are described by [Casadei et al.](#)  
188 [\(2021\)](#).

189 The concentrations used for calibration curves were 0.05, 0.10, 0.15, 0.20, 0.25, 0.5, 1.00,  
190 1.50, 2.00, 2.50, 5.00, and 10.00 mg/kg for the compounds included in SM-A. The calibration curves  
191 for the compounds in SM-B were the same but adding three new points (15.00, 20.00, and 25.00  
192 mg/kg), since most of these compounds were present in VOO at higher concentration.

## 193 *2.8 Peer inter-laboratory validation of the method*

194 The parameters considered were those in accordance with ISO 78-2 and ISO 5725 (ISO, 2016,  
195 2019): repeatability, reproducibility, linearity, recovery, precision, limits of detection (LOD) and  
196 quantification (LOQ), which were compared in order to have a peer inter-laboratory validation of the  
197 method. This study was carried out for each of the 18 quantified VOCs.

### 198 *2.8.1 Linearity*

199 Linearity was evaluated using the calibration curve for each VOC (section 2.7). The regression  
200 coefficient ( $R^2$ ) was considered for each calibration curve, built as linear regression passing through  
201 the origin of the axes.

### 202 *2.7.2 Repeatability*

203 The repeatability of the method was studied in terms of intra-day precision with a single  
204 operator and instrument in each of the laboratories. With this purpose, one L sample (with rancid as  
205 main perceived defect) selected from the 15 samples was analyzed seven times in a single batch; the  
206 relative standard deviation (RSD%) was calculated for each of the 18 analytes.

### 207 *2.7.3 Reproducibility*

208 For reproducibility, the study was based on the analysis of the 15 samples. These samples  
209 were analyzed in duplicate by the five laboratories. The relative standard deviation of the  
210 concentrations provided by the involved labs was calculated.

211

#### 212 2.7.4 Recovery

213 Recovery was calculated by analyzing the two standard mixtures, SM-A and SM-B, diluted  
214 in refined olive oil to reach 5 mg/kg. For each of the 18 analytes, the following formula was applied:

$$215 R_{ap} = \frac{C}{C_{ref}} \times 100$$

216 Where  $R_{ap}$  was the apparent recovery,  $C$  is the concentration determined with QM1, QM2 or  
217 QM3 (see section 2.6), and  $C_{ref}$  is the actual concentration calculated from the exact weights in the  
218 dilution of SM-A and SM-B to reach the target concentration of 5 mg/kg.

#### 219 2.7.5 Precision associated with the internal standard

220 To calculate the precision associated with the IS, the relative standard deviation (RSD) of the  
221 chromatographic area of the IS (4-methyl-2-pentanol) determined in the repeatability study (see  
222 section 2.7.2) was used. In fact, the precision should not only consider variability in the instrumental  
223 measurement, but also in the addition of the IS. The precision ( $RSD\%_{Area\ IS}$ ) was calculated using the  
224 formula:

$$225 RSD\%_{Area\ IS} = \frac{\delta_{Area\ IS}}{\bar{X}_{Area\ IS}} \times 100$$

226 Where  $\delta_{Area\ IS}$  is the standard deviation of the chromatographic areas assigned to the IS and  $\bar{X}_{Area\ IS}$   
227 is the average of these areas.

#### 228 2.7.6 Limits of detection (LODs)

229 LOD was defined as the minimum amount or concentration of each compound that can be  
230 reliably detected. Since several procedures to calculate LOD and LOQ are available in the literature,  
231 in this investigation different calculation methods were applied, all being based on the slope of the  
232 calibration curves ( $m$ ) and the standard errors of the regression ( $SE_{regression}$ ) and intercept ( $SE_{intercept}$ )

233 (Desimoni & Brunetti, 2015; Shrivastava & Gupta, 2011) through the following equations  
234 (henceforth, calculation methods 1-4):

235 1)  $LOD = 3.3 \times (SE_{\text{regression}}/m_{QM1})$ , using the ratio  $Area_{\text{Analyte}}/Area_{\text{IS}}$  as the variable Y of the regression  
236 and where SE is the standard error of the regression.

237 2)  $LOD = 3.3 \times (SE_{\text{intercept}}/m)$ , using the ratio  $Area_{\text{Analyte}}/Area_{\text{IS}}$  as the variable Y of the regression with  
238 intercept different from zero.

239 3)  $LOD = 3.3 \times (SE_{\text{intercept}}/m)$ , using the  $Area_{\text{Analyte}}$  as the variable Y of the regression with intercept  
240 different from zero.

241 These three methods were applied in the five laboratories to extract the LODs. Additionally, a fourth  
242 method (henceforth calculation method 4) based on the following equation was applied:

243 4)  $LOD = 3.3 \times (\delta_{\text{Areas}}/m_{QM1})$ , where  $\delta_{\text{Areas}}$  (standard deviation) is referred to three replicated areas at  
244 low concentration (0.05 mg/kg).

#### 245 *2.7.7 Limits of determination or quantification (LOQs)*

246 LOQ was calculated through the same calculation methods applied for LOD, but applying a  
247 factor of 10 instead of 3.3, both based on the calibration curves (see methods 1-4 listed in the section  
248 2.7.6) and the additional calculation of S/N. In the latter, a S/N of 10 is generally accepted to be  
249 sufficient to allow for quantification of the analyte.

#### 250 *2.8 Data processing and statistical analysis*

251 Data processing and calculations were carried out with Microsoft® spreadsheet program 2016  
252 (Microsoft Corp., Redmond, WA). Outlier detection was performed with Grubbs' test (Grubbs,  
253 1950). Analysis of variance ( $p < 0.05$ ) was carried out with Statistica (StatSoft Inc., Tulsa, OK).

### 254 **3. Results and Discussion**

255 The performance of the method was assessed through evaluation of several parameters  
256 (Aparicio-Ruiz et al., 2021), as explained in the following paragraphs. Moreover, a discussion was  
257 carried out that focused on comparison of results with those related to the parallel SPME-GC-FID  
258 approach (Casadei et al., 2021) with the view to evaluate the advantages, disadvantages and/or  
259 opportunities offered by the two detectors.

260 In assessment of these parameters, data obtained by the laboratories were reported in an Excel  
261 file to avoid errors and ensure that they were computed using the same procedure.

### 262 3.1 Linearity

263 Linearity was studied for the two types of calibration curves described in section 2.7 (QM1  
264 and QM2). The study of regression performance (mainly  $R^2$  coefficient and typical error) for these  
265 two quantification strategies allowed assessment of the effect of the IS in linearity, since both  
266 quantification methods differs in the use of the IS to correct the calibration curves. Table 3 shows the  
267 mean values of the  $R^2$  for the 18 volatile compounds reported by the five labs.  $R^2$  coefficients were  
268 higher than 0.94 for the 18 selected volatile compounds. The coefficients provided by the labs were  
269 homogeneous and no large differences between them were detected. Thus, the standard deviations of  
270  $R^2$  for the five labs had a maximum of 0.058 and 0.072 for QM1 and QM2 respectively. The  $R^2$  data  
271 were significantly higher ( $p>0.05$ ) for QM1 for ethyl acetate, ethanol, ethyl propanoate, 3-methyl-1-  
272 butanol, while  $R^2$  were higher for QM2 in the case of (*E*)-2-heptenal, 6-methyl-5-hepten-2-one,  
273 nonanal, (*E,E*)-2,4-hexadienal, and pentanoic acid. However, the effect of the IS was more evident in  
274 the improvement of linearity in QM1 for the aforementioned compounds. Figure S1 shows the  
275 calibration curves of ethyl propanoate and (*E,E*)-2,4-hexadienal as examples of two compounds in  
276 which the IS had an evident effect on linearity. Although these are two extreme cases that were not  
277 seen in all the labs and the effect of IS on linearity was not always so obvious, the mean  $R^2$  (Table 3)  
278 showed a clear effect of linearity for these two compounds. Thus, in the case of ethyl propanoate, the  
279 correction by the IS (QM1) produced a better linearity ( $R^2$  for QM1 and QM2 were 0.994 and 0.939,

280 respectively), while in the case of (*E,E*)-2,4-hexadienal, better linearity was obtained when the  
281 calibration was made without the correction applied by the IS ( $R^2$  for QM1 and QM2 were 0.975 and  
282 0.997, respectively).

283 The compound that provided the worst linearity in terms of  $R^2$  was (*E*)-2-decenal ( $R^2$  for QM1  
284 and QM2 were 0.942 and 0.966). On the other hand, the best linearity ( $R^2 > 0.990$ ) was observed for  
285 (*E*)-2-hexenal, acetic acid, 1-hexanol, ethyl propanoate, hexanal, octane, and 3-methyl-1-butanol.

286 The typical errors and slopes of each compound were also studied in the case of QM1, where  
287 the concentration is calibrated against the ratio of  $\text{Area}_{\text{Analyte}}/\text{Area}_{\text{IS}}$ , and the latter ratio allows  
288 comparison between labs and instruments. The slopes for each compound are shown in [Table S2](#) and  
289 [Figure S2](#). The slope was particularly high for ethyl acetate and ethyl propanoate, with a mean slope  
290 of 0.666 and 0.508, respectively ([Table S2](#)). However, the standard deviation of these mean slopes  
291 (0.655 and 0.552 for ethyl acetate and ethyl propanoate, respectively) demonstrates the wide variety  
292 between labs. Thus, for example, [Figure 2](#) shows the calibration curves of the five labs for ethyl  
293 propanoate. The different slopes can indicate the different sensitivities of the MS detector for this  
294 compound. Excluding octane, ethanol and acetic acid, for the remainder of the compounds, the slope  
295 values were lower than 0.1 ([Table S2](#)). In terms of typical error, the highest mean errors were found  
296 for ethyl acetate and ethanol (0.231 and 0.184, respectively), with also a large difference between  
297 labs.

### 298 3.2 Repeatability

299 The repeatability of the method was studied for each of the compounds quantified by each one  
300 of the three quantification methods (QM1, QM2 and QM3). [Table 4](#) shows the repeatability expressed  
301 as mean RSD%. Considering the results for QM1, the volatile compounds with RSD% higher than  
302 10% were ethyl propanoate, nonanal, and (*E*)-2-decenal. The RSD% value for the latter compound  
303 was particularly high (17.23%), probably due to the low concentration in the sample studied (0.002  
304 mg/kg). The average RSD% for the 18 compounds was 7.60%, although it was 6.16% when the three



305 aforementioned compounds were omitted. Regarding the other two quantification methods, QM2 and  
306 QM3, the RSD% values were generally lower compared with QM1. However, significant differences  
307 were found only for the acids (acetic, propanoic and pentanoic acids) between the RSD% values from  
308 QM1 and QM2, in (*Z*)-3-hexenyl acetate and (*E*)-2-decenal between the RSD% values from QM1  
309 and QM3, and in the (*Z*)-3-hexenyl acetate and 1-hexanol between the RSD% values from QM2 and  
310 QM3 (Table 4).

311 The RSD% values of the duplicates of the 15 VOOs were also examined to check if the  
312 repeatability RSD% shown in Table 4 agreed with the variability observed in the duplicates,  
313 considering that the 15 samples included a wide range of qualities and concentration values. These  
314 RSD% values are shown in Figure S3. The highest RSD% values corresponded to ethyl propanoate  
315 ( $8.38 \pm 7.58\%$ ), nonanal ( $14.18 \pm 13.82\%$ ), 1-octen-3-ol ( $11.20 \pm 10.36\%$ ), (*E,E*)-2,4-hexadienal  
316 ( $9.71 \pm 8.55\%$ ), (*E*)-2-decenal ( $10.83 \pm 8.31\%$ ), and pentanoic acid ( $12.32 \pm 11.85\%$ ). These results  
317 confirmed the lower repeatability for ethyl propanoate, nonanal and (*E*)-2-decenal.

### 318 3.3 Reproducibility

319 The reproducibility was studied by analyzing 15 samples in duplicate by each lab, including  
320 the three quality categories. Table 5 shows the mean RSD% for each VOC for the first quantification  
321 method (QM1). The concentration ranges determined by the labs for each sample are also shown in  
322 Table 5. Outliers were removed by Grubbs' test ( $\alpha = 0.05$ ). The higher RSD% values ( $> 40\%$ )  
323 corresponded to 6-methyl-5-hepten-2-one (43.20%), nonanal (46.05%), and (*E,E*)-2,4-hexadienal  
324 (63.46%). Octane (38.50%) and ethyl propanoate (38.96%) also showed RSD% close to 40%. In the  
325 case of ethyl propanoate, these values can be explained by the low concentration values ( $< 0.05$  in  
326 most cases). The lowest RSD% values ( $< 20\%$ ) were found for (*Z*)-3-hexenyl acetate (19.19%), 1-  
327 hexanol (13.26%), and acetic acid (17.47%). Table 5 shows the RSD% values when the quantification  
328 methods QM2 and QM3 were applied. The RSD% values for QM1 were generally lower compared  
329 with those found for QM2 and QM3. Thus, RSD% average values for the 18 compounds were

330 30.89%, 48.02% and 55.41%. The comparison of RSD% values for QM1 and QM2 revealed a  
331 correction effect of the IS when results from different labs are compared, while the intra-lab  
332 repeatability RSD% was similar or lower for QM2 in which no IS correction was applied (Table 5).  
333 The reproducibility RSD% values of QM1 were significantly lower ( $p < 0.05$ ) than the values obtained  
334 with QM2 for 10 of the 18 compounds: octane, ethyl acetate, 3-methyl-1-butanol, (*E*)-2-hexenal, (*Z*)-  
335 3-hexenyl acetate, (*E*)-2-heptenal, 6-methyl-5-hepten-2-one, 1-hexanol, propanoic acid, and  
336 pentanoic acid (Table 5). Regarding QM3, the RSD% values were also significantly higher than those  
337 obtained with QM1 for 8 compounds. These results highlight that QM1 was the best method in terms  
338 of reproducibility. However, recovery (section 3.4), among other parameters, is also another  
339 important criterion to be considered.

#### 340 3.4 Recovery

341 Table 6 shows the mean recovery values (%) for each of the selected volatile compounds  
342 obtained with the three quantification methods (QM1, QM2, and QM3). The recovery values derived  
343 from the ratio of the actual concentrations, obtained considering the exact weights in the dilution of  
344 SM-A and SM-B to reach the target concentration (5 mg/kg), with the calculated ones determined  
345 with the three quantification methods. The mean recovery values were 94%, 105% and 179% for  
346 QM1, QM2, and QM3, respectively. These results are comparable with the same values obtained in  
347 a parallel peer inter-laboratory validation work carried out with FID detector and three labs: 89%,  
348 115%, and 181% for QM1, QM2, and QM3, respectively (Casadei et al., 2021). From the three  
349 quantification methods, QM1 provided the best recovery (close to 100%) among the three calculation  
350 methods, followed by QM2. Thus, the mean recovery values ranged from 72% to 106% for QM1  
351 while they ranged from 71% to 150% for QM2. In another work, a method based on dynamic  
352 headspace thermal desorption (DHS-TD) combined to GC-MS was developed to identify and  
353 simultaneously quantify 51 VOCs in EVs and the recoveries obtained ranged from 50.9% to 113.9%  
354 (Reboredo-Rodríguez et al., 2012). However, this study was carried out with a different sampling and  
355 therefore the recovery values are not fully comparable (Oliver-Pozo et al., 2019). Following the

356 analysis of the results in the present study, QM2 showed better results for nonanal and acetic acid  
357 compared to QM1. These results point out that the IS exerted a negative effect by introducing more  
358 error in the quantification for these two compounds, while the use of IS reduced quantification errors  
359 in terms of accuracy in the remainder of the compounds. Nevertheless, a dependent analysis of  
360 variance ( $p < 0.05$ ) showed that there were no significant differences between the recovery values  
361 obtained with QM1 and QM2. In the case of QM3, a significant difference with respect to QM1 was  
362 observed for (*E*)-2-decenal. Furthermore, the high standard deviation for the recovery values obtained  
363 for QM3 for all the compounds points out the higher variation of the values between labs when this  
364 quantification methodology is applied. Thus, the standard deviation varied between 5-67% for QM1,  
365 11-80% for QM2, and 29-221% for QM3.

366 Analyzing the differences between compounds, and focusing on recovery values for QM1, the  
367 highest errors (difference of recovery values with respect to 100%) in quantification were observed  
368 for (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, 1-octen-3-ol, acetic acid, and nonanal and (*E*)-2-decenal,  
369 which were particularly noticeable in the latter compound. Thus, the deviation of 100% recovery in  
370 this compound was around 28% (Table 6), while in the other 5 compounds this error was always  
371 below 20%. With respect to the other compounds, the deviation from  $R_{ap} = 100\%$  was always lower  
372 than 10%. Only ethanol, ethyl propanoate, hexanal, (*E*)-2-heptenal, and 6-methyl-5-hepten-2-one  
373 were affected by a slight overestimation ( $R_{ap} > 100\%$ ), while the remainder were affected by  
374 underestimation ( $R_{ap} < 100\%$ ).

375 In general, the different recoveries obtained for the selected compounds can be partially  
376 explained by a low or higher adsorption on the fiber and by competition phenomena with other  
377 compounds that have a higher affinity for the fiber polymers (Oliver-Pozo, Aparicio-Ruiz, Romero,  
378 & García-González, 2015). These phenomena may influence the linearity of the calibration curves,  
379 especially when the compounds are present at high concentrations. With the aim of evaluating the  
380 impact on quantification of the possible lack of linearity at the points of high concentrations ( $>10$

381 mg/kg), the analytes were quantified again using a calibration curve at low concentrations (0.05-2.5  
382 mg/kg) and the recovery values were compared when the entire concentration range was used in the  
383 calibration (0.05-10.00/25.00 mg/kg) (Table 6). In the case of the recovery values calculated from  
384 QM3, no significant differences were observed when comparing the recoveries obtained from the two  
385 concentration ranges. The lack of a significant difference may be partially explained by the high  
386 variation of recovery values for QM3 between the 5 labs. This variation was shown by the standard  
387 deviation found for QM3 recoveries, which was higher compared with those for QM1 and QM2  
388 (Table 6). On the contrary, in the case of QM1, significantly different recovery values were obtained  
389 for ethyl acetate and (*E*)-2-decenal, whereas significant differences were found for octane, ethyl  
390 acetate, ethyl propanoate, propanoic acid and (*E*)-2-decenal for QM2. Regarding the mean of the  
391 mean recovery values, they were 94.23% and 129.80% for QM1 when the entire concentration range  
392 and the low concentration range were used respectively. These two values were 105.04% and  
393 100.99% for QM2 and 179.29% and 176.26% for QM3. These results show that the calibration with  
394 lower concentrations did not produce better results in general terms since significant differences were  
395 found for only some compounds.

### 396 *3.5 Precision associated with the IS*

397 Since the IS influences quantification, the RSD% of the chromatographic areas corresponding the IS  
398 was studied for each of the participant labs by analyzing the 15 samples for the reproducibility study  
399 (N = 15 for each lab). The RSD% ranged from 4.02% to 15.44% for the five labs, the mean RSD%  
400 being 9.66%. This error could be attributed to instrumental error or to competition phenomena in the  
401 absorption to the SPME fiber rather than to the human error by adding 0.1 g of the IS solution to the  
402 sample. A study made by adding 0.1g of this solution by one operator for 60 times (N = 60) revealed  
403 a RSD% value in the measured weights of only 0.66%. The lowest values of the IS chromatographic  
404 areas corresponded to L and V olive oils category in which high intensity of defects were identified  
405 and consequently the higher concentration of compounds can produce competition phenomena

406 (Oliver-Pozo et al., 2015). Thus, two samples coded as S5 and S15 (Table 5) were characterized with  
407 significantly lower values of IS chromatographic areas, and these two samples were two L oils with  
408 a high median of defect (5.2 and 5.4, respectively, for fusty/muddy sediment defect). Without these  
409 two samples, the average RSD% was 7.15% (ranging from 4.06% to 11.46%).

### 410 3.6 Limits of detection (LOD)

411 Three methodologies were studied to obtain the limits of detection in the calibration curves  
412 built by each of the VOCs. The first method (calculation method 1, section 2.7.6) used standard error  
413 of the regression and the calibration equations having an intercept forced to zero. The other two  
414 methods, referred to as calculation methods 2 and 3, used calibration equations having an intercept,  
415 and the standard deviation of this intercept was used in the calculation of the LOD. Method 2 used  
416 the chromatographic area of the analyte divided by the area of the IS as instrument output, while  
417 method 3 used the chromatographic area of the analyte. The objective of applying different methods  
418 was to check the consistency of the LOD obtained through different procedures and to check which  
419 results best matched with the actual observations of the signals at low concentrations (Aparicio-Ruiz  
420 et al., 2018). The LOD values calculated with these methods are shown in Table 7 as means and  
421 ranges of the values obtained from the laboratories involved. The values were > 0.10 mg/kg for all  
422 compounds. Method 1 produced higher values than methods 2 and 3. Thus, the LOD obtained from  
423 calculation method 2 ranged from 0.10 to 0.59 mg/kg, while the LODs from method 1 were higher  
424 than 1.00 mg/kg for 9 compounds.

425 The highest values of LODs in the three methods were found for hexanal, 1-hexanol, 1-octen-  
426 3-ol, (E,E)-2,4-hexadienal, acetic acid, and (E)-2-decenal (e.g. > 1.5 mg/kg for calculation method  
427 1). The lowest values were found for octane, ethyl acetate, ethyl propanoate, 3-methyl-1-butanol, and  
428 propanoic acid (e.g. < 0.65 mg/kg for calculation method 1). However, it was observed that  
429 concentrations which were lower than the calculated LODs produced clearly detectable signals as  
430 observable peaks in the chromatogram with measurable chromatographic areas. Thus, the LOD

431 values obtained with these methods did not match the perceived signals when analyzing compounds  
432 in the low concentration range of the calibration curve (0.05-0.25 mg/kg). In the low concentrations,  
433 the signals were always detected and linearity was observed. [Table S3](#) shows the regression  
434 coefficients ( $R^2$ ) when low concentrations were considered (0.05, 0.10, 0.15, 0.20, 0.25 mg/kg). All  
435 compounds showed  $R^2$  values  $>0.90$  in this range of the calibration, except for nonanal and (*E*)-2-  
436 decenal (0.613 and 0.629, respectively), since they were barely detected at low concentration (0.05  
437 mg/kg) by three of the five laboratories. On the contrary, two labs obtained  $R^2$  values  $> 0.95$  for these  
438 two compounds. In addition, the calculated standard deviation of the  $R^2$  presented low values, being  
439  $< 0.11$  for all the compounds except nonanal and (*E*)-2-decenal (0.436 and 0.431, respectively). These  
440 results show that the response of the detector for nonanal and (*E*)-2-decenal may differ depending on  
441 the characteristics of the mass detector. The low LODs in these two compounds is also affected by  
442 the low adsorption to the SPME fiber compared with other compounds. Thus, [Figure S4](#) shows the  
443 chromatograms of SMA and SMB ([Table 2](#)) diluted at a concentration of 20 mg/kg. Nonanal and (*E*)-  
444 2-decenal showed a chromatographic area that were 10 times lower than the other compounds. [Table](#)  
445 [S3](#) also shows the values of the slope and intercept when a regression equation is built with the low  
446 concentration range. The mean values of the slope ranged from 0.001 to 0.959, which shows a  
447 different sensitivity of the detector depending on the compounds. On the other hand, the intercept  
448 values were close to zero in all cases, ranging from -0.033 to 0.014, pointing out a lack of impurities  
449 or noise.

450 The results described above illustrate the need to calculate LOD values that are in accordance  
451 with observations when the analytes are analyzed at low concentrations. Thus, an additional method  
452 (calculation method 4) based on the standard deviation of the areas for three replicates of the analyses  
453 of the analytes at low concentration (0.05 mg/kg) was applied. This methodology provided more  
454 representative values when it was applied in the peer validation study for SPME-GC-FID method  
455 ([Casadei et al., 2021](#)). The LOD values were in the range 0.01-0.18 mg/kg. The lowest LODs (0.01

456 mg/kg) corresponded to octane, 3-methyl-1-butanol, (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, 1-hexanol,  
457 1-octen-3-ol, (*E,E*)-2,4-hexadienal, acetic acid, and propanoic acid, while the highest LOD (0.18  
458 mg/kg) corresponded to (*E*)-2-decenal. The comparison of these LOD values and the concentrations  
459 calculated in the 15 samples (Table 5) revealed that many samples showed concentration values lower  
460 than the LODs in the case of ethyl propanoate, (*E*)-2-decenal and pentanoic acid. However, these  
461 problems did not fully explain the reproducibility RSD% for these compounds, since their values  
462 (38.96, 36.65, 27.11% respectively when QM1 is applied) were not the highest (Table 5).

### 463 3.7 Limits of determination or quantification (LOQ)

464 The LOQ values calculated with the three methods are shown in Table 8. The values were  
465 high (> 1.0 mg/kg in most of the cases) and did not correspond with the clearly distinguishable signals  
466 and high linearity observed in the chromatographic areas when the analyte was present at low  
467 concentrations (< 0.25 mg/kg) (Table S3). In the case of method 1, the LOQs were around 5 mg/kg  
468 for hexanal, 1-hexanol, 1-octen-3-ol, acetic acid and (*E*)-2-decenal. However, with calculation  
469 method 4, LOQs were in the range of 0.01-0.53 mg/kg. Considering this method, the lowest LOQs  
470 (<0.03 mg/kg) corresponded to 1-hexanol, (*Z*)-3-hexenyl acetate, propanoic acid, octane, (*E*)-2-  
471 hexenal, and 1-octen-3-ol. The highest LOQs (> 0.07 mg/kg) corresponded to ethyl propanoate,  
472 hexanal, ethyl acetate, ethanol, nonanal, pentanoic acid and (*E*)-2-decenal. The concentrations  
473 calculated in the 15 samples were lower or close to the LOQ in most samples for ethyl propanoate,  
474 1-octen-3-ol, (*E*)-2-decenal and pentanoic acid. However, as stated above, this did not seem to affect  
475 the RSD% values for reproducibility (Table 5). On the contrary, the highest RSD% value (63.46%  
476 when QM1 was applied) was found for (*E,E*)-2,4-hexadienal (Table 5), which could be explained by  
477 the fact that its concentrations was close to the LOQ limit, even if all the concentrations were higher  
478 than the LOD. This could lead to some difficulties in integration and result in higher errors.

479

480

### 481 3.8 Comparative study of validations using SPME-GC-FID and SPME-GC-MS methods

482 A comparative study of the SPME-GC-FID method carried out by three labs (Casadei et al.,  
483 2021) and the present SPME-GC-MS (applied by five labs) was made considering the values of the  
484 parameters studied in each validation for the set of 18 VOCs. Both studies were carried out on the  
485 same samples and with exactly the same procedure.

486 In terms of linearity, the mean values of  $R^2$  were slightly higher for MS (0.983) than for FID  
487 (0.977). In addition, higher  $R^2$  values were observed using QM1 with respect to QM2, both in FID  
488 and MS, which indicates a general improvement of the calibration results when the IS is added and  
489 used in quantification of the concentration of compounds. The VOCs that showed the highest linearity  
490 in their calibrations were ethyl propanoate and 3-methyl-1-butanol for FID ( $R^2 = 0.998$ ) and octane,  
491 hexanal and 3-methyl-1-butanol for MS ( $R^2 = 0.996$ ). The lowest linearity was observed for (*E*)-2-  
492 heptenal in FID ( $R^2 = 0.936$ ) and for (*E*)-2-decenal in MS ( $R^2 = 0.942$ ). In general terms, compounds  
493 presenting high  $R^2$  values for the labs that used FID matched with those that presented high linearity  
494 for the labs using MS. The same was observed for compounds with less linearity.

495 Regarding repeatability, MS presented lower mean RSD% values in each of the three QMs  
496 applied (7.60% for QM1, 6.00% for QM2 and 5.70% for QM3 in MS; compared to 11.52%, 8.18%  
497 and 9.65% in FID, respectively). Therefore, QM1 gave the highest mean RSD% value, both in FID  
498 and MS, and the best repeatability was obtained by applying QM2 in FID and QM3 in MS. The  
499 RSD% values considering the three QMs ranged between 3.60% and 15.62% for FID and between  
500 2.21% and 17.23% for MS. Thus, the performance of the methods in terms of repeatability was similar  
501 when using the two detectors. The VOCs that showed the best repeatability (lower mean RSD% value  
502 considering the three QMs) were acetic acid and propanoic acid with FID (5.18% and 5.74%,  
503 respectively) and (*Z*)-3-hexenyl acetate and (*E*)-2-hexenal with MS (3.76% and 3.83%, respectively).  
504 Ethyl propanoate and 1-octen-3-ol had the highest mean values of RSD% in FID (13.80% and



505 13.29%, respectively), whereas ethyl propanoate, again, and hexanal (11.37 % and 10.14%,  
506 respectively) had the worst repeatability in MS validation.

507           Considering the reproducibility of the method, both for FID and MS showed similar or better  
508 RSD% values with QM1 compared with QM2 and QM3. However, the advantage of using QM1 is  
509 more evident in the method using MS. Thus, the mean RSD% values of the 18 VOCs for QM1, QM2  
510 and QM3 were 38.79%, 39.18% and 37.66% for FID and 31.77%, 48.02% and 55.41% for MS,  
511 respectively. On the other hand, of the 18 selected compounds, the use of IS in the quantification  
512 showed to have a positive effect in reproducibility (lower RSD% for QM1 compared to QM2) in 7  
513 compounds in FID and 16 compounds in MS. Considering only QM1, the mean RSD% for the 18  
514 VOCs quantified was lower in MS than in FID, ranging between 12.05% (octane) and 121.99% (ethyl  
515 propanoate) for FID; and between 13.26% (1-hexanol) and 63.46% ((*E,E*)-2,4-hexadienal) for MS.  
516 However, excluding this anomalous value of RSD% in ethyl propanoate in the validation with FID,  
517 the mean RSD% for the rest of VOCs would be 32.59% and the maximum value of RSD% would be  
518 48.06% for 1-hexanol. For 6 compounds (octane, ethyl acetate, 3-methyl-1-butanol, nonanal, (*E,E*)-  
519 2,4-hexadienal, and propanoic acid), the RSD% value was lower in the method using FID compared  
520 to MS, although 3 compounds (octane, ethyl acetate, (*E,E*)-2,4-hexadienal) had a clear difference,  
521 with the RSD% for FID being approximately one half. For the rest of compounds (12), the RSD%  
522 were lower for MS, and in 3 (ethyl propanoate, 1-hexanol, acetic acid) the RSD% was the half as low  
523 or even less compared to the method using FID.

524           When comparing the recovery between the two methods, mean values closer to 100% were  
525 observed in the laboratories that used MS for QM1 and QM2 (94% and 105% with MS vs. 89% and  
526 115% with FID, respectively). QM3 had very high recovery values in both validations (mean values  
527 of 181% and 179% for FID and MS, respectively). Even though, as stated, the quantification with  
528 QM1 provided very similar average recovery results compared to QM2 in both validations, the mean  
529 deviation from 100% was substantially lower for QM1 in the laboratories using MS (7.70% applying

530 QM1 vs. 16.40% with QM2). The compound with the best recovery using QM1 was 6-methyl-5-  
531 hepten-2-one in FID (99%), and 3-methyl-1-butanol and 1-hexanol (100%) in MS. The compound  
532 with deviation greater from 100% was (*E*)-2-decenal, in both FID (160%) and MS (72%).

533 Precision, expressed as the RSD% of the chromatographic areas corresponding to the IS (4-  
534 methyl-2-pentanol) ranged from 4.52% to 9.65% (mean 7.56%) in the validation with FID. Using  
535 MS, the RSD% ranged from 4.02% to 15.44% for the five labs, with a mean RSD% of 9.66%. As  
536 observed, the obtained values were low, which suggested good precision for both FID and MS  
537 validations.

538 The LOD values of the 18 VOCs was calculated using 4 different methods. In both the  
539 validations with FID and MS, calculation method 4 had lower and more representative values for this  
540 parameter with respect to the other methods, and thus was the method of choice. In both cases, the  
541 values coincided with the visual analysis of peaks for most of the VOCs in the calibration  
542 chromatograms. On the other hand, the laboratories that used MS obtained mean values of LOD that  
543 were lower than the laboratories using FID (0.03 mg/kg and 0.08 mg/kg with calculation method 4,  
544 respectively). The compound with the lowest LOD in both validations was 1-hexanol (<0.005 mg/kg  
545 in FID and 0.01 mg/kg in MS), while the one with the highest value for this parameter was (*E*)-2-  
546 decenal (0.64 mg/kg in FID and 0.18 mg/kg in MS), for both types of detectors.

547 For the LOQ, the same conclusions as for the LOD were reached since the difference between  
548 the two limits is only a factor of 3. In fact, the LOQ values were about 3 times greater than those  
549 obtained in the calculation of the LOD, ranging between 0.01 mg/kg (1-hexanol) and 1.93 mg/kg  
550 ((*E*)-2-decenal) in the validation with FID and between 0.01 mg/kg and 0.53 mg/kg (for the same two  
551 VOCs) in validation with MS.

552

553

#### 554 4. Conclusions

555 The purpose of this investigation was the peer validation study of a SPME-GC-MS method  
556 for analysis of selected VOCs to support sensory analysis in quality control of VOOs. This represents  
557 a further step forwards in the quali-quantitative evaluation of diagnostic volatile markers under the  
558 same analytical conditions of a method using FID as a detector (Casadei et al., 2021). This work was  
559 useful to make the entire process of full validation more robust and effective also thanks to the  
560 organization, within the OLEUM project, of a hands-on training workshop that focused on this  
561 method, and pre-trials as collaborative inter-laboratory experiments. After that, the proficiency of the  
562 method was also evaluated through a proper inter-laboratory trial with the active involvement of  
563 several external laboratories with a consolidated expertise in the olive oil analytical sector.

564 From this peer inter-laboratory study, method performance parameters obtained in each  
565 laboratory were investigated, compared and discussed with the aim to highlight similarities and  
566 eventual differences, as well as to calculate mean values and dispersion of the results. The  
567 quantification of the selected VOCs was carried out on the same samples by applying three different  
568 quantification methods (QMs): from analysis of all the dataset it turned out that the most promising  
569 method was QM1 using a calibration based on the IS and the external calibration curve ( $A_{\text{Analyte}}/A_{\text{IS}}$   
570 vs.  $C_{\text{Analyte}}$ ). Although QM1 showed slightly worse repeatability than the other methods, it had better  
571 accuracy and reproducibility. This finding was also observed for the FID method, even if with MS it  
572 was more evident. In general, satisfactory results were obtained for linearity, recovery, precision and  
573 repeatability parameters, although reproducibility has a rather high RSD% (>40%) for some  
574 compounds (ethyl propanoate, 6-methyl-hepten-2-one, and (E,E)-2,4-hexadienal).

575 This study compared the performance characteristics of the method when applied with FID or  
576 MS. Given that these two options provide advantages and disadvantages, and that they are  
577 alternatively available in the labs working in olive oil analysis, knowledge on their performance is  
578 needed. Only at the end of a full validation process with the involvement of a large number of

579 laboratories participating on a voluntary basis, it will be possible to conduct a study aimed at  
580 individuating the concentration ranges of variability, as well as a proposal of limits, for the selected  
581 volatile compounds (especially those related to sensory defects) in relation to the different quality  
582 grades of VOOs. Moreover, also considering the pros that - for the samples analyzed herein - the  
583 sensory evaluation was performed by 6 different panels, the concentrations obtained could be related  
584 with the presence of sensory defects or positive attributes (fruity), thus being useful to define the  
585 ranges/limits for the selected markers in order to support the panel test.

586

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592

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757

758 **Figure captions**

759 **Figure 1.** Chromatogram of volatile compounds of a lampante olive oil and a virgin olive analysed  
760 by SPME-GC-MS. The correspondence of the codes with the volatile compounds is shown in Table  
761 1.

762 **Figure 2.** Calibration curves of ethyl propanoate built for the quantification method 2 (QM2).

Table 1. Selected volatile compounds, CAS numbers, purities of chemical standards, linear retention times (LRT), characteristic m/z in the mass spectra and sensory defects or positive attribute to which they are related.

Code	Volatile compound	CAS number	Purity of the chemical standard (%) <sup>a</sup>	LRI <sup>b</sup>	SM <sub>x</sub> <sup>c</sup>	Characteristic m/z	Related defect/attribute <sup>d</sup>
1	Octane	111-65-9	99.7	802 ± 1.85	A	85	Fusty/muddy sediment
2	Ethyl acetate	141-78-6	99.8	899 ± 0.84	A	43	Winey-vinegary
3	Ethanol	64-17-5	99.9	933 ± 1.06	B	31, 45	Winey-vinegary, fusty/muddy sediment
4	Ethyl propanoate	105-37-3	99.7	954 ± 7.23	A	57	Frostbitten olives
5	Hexanal	66-25-1	98	1080 ± 8.02	B	44	Rancid
6	3-Methyl-1-butanol	123-51-3	98.5	1210 ± 4.53	A	55, 70	Fusty/muddy sediment
7	( <i>E</i> )-2-Hexenal	6728-26-3	97.0	1215 ± 9.18	B	69, 83	Fruity
8	( <i>Z</i> )-3-Hexenyl acetate	3681-71-8	98.0	1312 ± 4.96	B	67, 82	Fruity
9	( <i>E</i> )-2-Heptenal	18829-55-5	95	1321 ± 10.08	A	83	Musty-humid-earthy, rancid
10	6-Methyl-5-hepten-2-one	110-93-0	97.0	1337 ± 10.00	A	108	Fusty/muddy sediment
11	1-Hexanol	111-27-3	99.9	1356 ± 4.79	B	56	Fruity
12	Nonanal	124-19-6	95	1392 ± 9.21	B	98	Rancid
13	1-Octen-3-ol	142-83-6	98.0	1453 ± 6.70 <sup>e</sup>	B	81	Musty-humid-earthy
14	( <i>E,E</i> )-2,4-Hexadienal	3391-86-4	95.0	1401 ± 10.71 <sup>e</sup>	A	57	Rancid
15	Acetic acid	64-19-7	99.8	1475 ± 35.27	B	60	Winey-vinegary
16	Propanoic acid	79-09-4	99.8	1547 ± 46.54	A	74	Fusty/muddy sediment, musty-humid-earthy
17	( <i>E</i> )-2-Decenal	3913-81-3	95.0	1644 ± 10.39	A	70	Rancid
18	Pentanoic acid	109-52-4	99.8	1759 ± 32.92	A	60, 73	Rancid

<sup>a</sup> Minimum purity as expressed by the supplier.

<sup>b</sup> LRI: Linear Retention Index, Relative Retention Time indicative parameter. Mean ± error from two labs that reported the results (UNIUD and UNIPG).

<sup>c</sup> SM: Standard mixture containing each volatile compound (SM-A: low concentration range 0.05-10.00 mg/kg; SM-B: high concentration range 0.20-25.00 mg/kg).

<sup>d</sup> Main perceived defect/attribute when the volatile compound is at high concentrations (above its odor threshold). Some compounds may be related to more than one defect/attribute.

<sup>e</sup> The order of these two compounds may be altered depending on the column brand and/or column film thickness.

Table 2. Characteristics of the GC-MS instruments used in each lab during the peer inter-laboratory validation study.

	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5
<b>GC Instrument (Equipment)</b>	7890B, Agilent Technologies <sup>1</sup>	78900A, Agilent Technologies <sup>1</sup>	7890B, Agilent Technologies <sup>1</sup> , equipped with a "Multimode Injector" (MMI) 7693A	6890N, Agilent Technologies <sup>1</sup>	HP6890, Agilent Technologies <sup>1</sup>
<b>Autosampler</b>	PAL RSI 85, CTC Analytics AG <sup>2</sup>	Combipal, CTC Analytics <sup>2</sup>	PAL3 RSI 120, CTC Analytics AG <sup>2</sup>	Combi-PAL, CTC Analytics AG <sup>2</sup>	MPS (MultiPurpose Sampler), GERSTEL GmbH & Co.KG <sup>3</sup>
<b>GC column</b>	DB-WAX, Agilent J&W <sup>1</sup> , CA. 60 m; I.D. 0.25 mm; film thickness 0.25 $\mu$ m	ZB-WAX Zebron, Phenomenex <sup>4</sup> , 60 m x 250 $\mu$ m x 0.25 $\mu$ m,	HP-INNOWax, Agilent Technologies <sup>1</sup> , 60 m; i.d. 0.25 mm; film thickness 0.25 $\mu$ m	Supelcowax-10, Supelco <sup>5</sup> , 60 m; I.D. 0.25 mm; film thickness 0.25 $\mu$ m.	DB-WAX Ultra Inert, Agilent J&W <sup>1</sup> , length 60 m, i.d. 0.25 mm; film thickness 0.5 $\mu$ m
<b>MS instrument (equipment)</b>	5977A, Agilent Technologies <sup>1</sup> , single quadrupole mass spectrometer,	5975C, Agilent Technologies <sup>1</sup> , single quadrupole mass spectrometer,	5977B, Agilent Technologies <sup>1</sup> , single quadrupole mass spectrometer with EI Extractor (XTR) source	5975C, Agilent Technologies <sup>1</sup> , inert XL quadrupole analyser	MSD5975, Agilent Technologies <sup>1</sup> , single quadrupole mass spectrometer
<b>MS database</b>	NIST v14 <sup>6</sup>	NIST MS Search 2.0 <sup>6</sup>	NIST v14 <sup>6</sup>	Wiley <sup>6</sup>	NIST v14 <sup>6</sup>
<b>GC-MS Interface Temp.</b>	280°C	275 °C	260 °C	280°C	220°C
<b>Ion source temperature</b>	175°C	230°C	200°C	230°C	200°C
<b>Mass range m/z</b>	31-350 m/z	30-300 m/z	25–350 m/z	35-300 m/z	29-350 m/z
<b>Quadrupole temperature</b>	150°C	150°C	190°C	150°C	150°C
<b>Scan rate</b>	1.6 scans/s	5.1 scan/s	4.3 scan/s	5.1 scans/s	2.0 scans/s

Note: <sup>1</sup>, Agilent Technologies, Santa Clara, CA, USA ; <sup>2</sup>, CTC Analytics AG, Zwingen, Switzerland; <sup>3</sup>, GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany; <sup>4</sup>, Torrance, CA, USA; <sup>5</sup>, Bellefonte, PA, USA; <sup>6</sup>, Gaithersburg, MD; <sup>7</sup>, Hoboken, NJ, USA.

Table 3. Linearity expressed as  $R^2$  (mean and standard deviation) computed from the calibration curves used in the quantification methods 1 and 2 (QM1, QM2) for the 18 volatile compounds.

<b>Volatile compounds</b>	<b>QM1</b>	<b>QM2</b>
Octane	0.996±0.003	0.966±0.038 <sup>a</sup>
Ethyl acetate	0.982±0.023 <sup>a</sup>	0.906±0.078 <sup>a</sup>
Ethanol	0.984±0.011 <sup>a</sup>	0.953±0.047 <sup>a</sup>
Ethyl propanoate	0.994±0.008	0.939±0.053 <sup>a</sup>
Hexanal	0.996±0.003	0.980±0.021
3-methyl-1-butanol	0.996±0.002	0.941±0.068 <sup>c</sup>
( <i>E</i> )-2-Hexenal	0.990±0.009 <sup>b</sup>	0.994±0.007 <sup>b</sup>
( <i>Z</i> )-3-Hexenyl acetate	0.987±0.012 <sup>b</sup>	0.992±0.006 <sup>b</sup>
( <i>E</i> )-2-Heptenal	0.976±0.027 <sup>b</sup>	0.997±0.001
6-Methyl-5-hepten-2-one	0.975±0.025 <sup>b</sup>	0.997±0.001
1-Hexanol	0.993±0.006	0.992±0.005
Nonanal	0.976±0.024	0.990±0.007
1-Octen-3-ol	0.983±0.019	0.993±0.005
( <i>E,E</i> )-2,4-Hexadienal	0.975±0.027 <sup>d</sup>	0.997±0.002
Acetic acid	0.993±0.005	0.989±0.011
Propanoic acid	0.983±0.028 <sup>b</sup>	0.995±0.005
( <i>E</i> )-2-Decenal	0.942±0.057 <sup>b</sup>	0.966±0.025 <sup>b</sup>
Pentanoic acid	0.969±0.032 <sup>b</sup>	0.993±0.008 <sup>b</sup>

<sup>a</sup> Certain saturation at high concentrations in data provided by some of the involved labs.

<sup>b</sup> Certain lower sensitivity (lower slope) at low concentrations in data provided by some of the involved labs.

Table 4. Repeatability expressed as mean RSD%.

Volatile compounds	RSD% (Mean±SD)		
	QM1	QM2	QM3
Octane	6.77±4.33 <sup>a</sup>	7.95±4.11	6.47±4.91
Ethyl acetate	6.99±3.49	4.77±0.21	5.75±4.02
Ethanol	9.51±2.72	6.21±2.14	6.52±1.94
Ethyl propanoate	15.27±15.87 <sup>a</sup>	15.55±15.63	15.13±17.34
Hexanal	5.49±3.67	4.84±2.00	4.53±1.94
3-Methyl-1-butanol	5.09±1.80	5.63±2.58	2.88±2.44
( <i>E</i> )-2-Hexenal	4.15±1.74	2.99±0.40	2.21±1.30
( <i>Z</i> )-3-Hexenyl acetate	5.23±0.55 <sup>c</sup>	4.86±0.84 <sup>d</sup>	3.11±0.61 <sup>cd</sup>
( <i>E</i> )-2-Heptenal	5.38±0.76	4.75±4.23	3.31±3.61
6-Methyl-5-hepten-2-one	5.05±1.17	5.82±0.89	4.40±0.07
1-Hexanol	3.89±1.46	4.12±0.72 <sup>d</sup>	2.39±0.34 <sup>d</sup>
Nonanal	11.84±7.33 <sup>a</sup>	9.89±3.96	7.36±9.39
1-Octen-3-ol	6.98±1.59	5.40±0.98	5.84±3.03
( <i>E,E</i> )-2,4-Hexadienal	8.51±2.99	4.20±0.72	6.79±5.13
Acetic acid	7.87±0.47 <sup>b</sup>	3.48±2.59 <sup>b</sup>	5.48±3.09
Propanoic acid	5.70±0.19 <sup>b</sup>	2.35±1.56 <sup>b</sup>	3.32±2.08
( <i>E</i> )-2-Decenal	17.23±5.08 <sup>c</sup>	12.00±2.77	13.86±5.10 <sup>c</sup>
Pentanoic acid	5.83±0.27 <sup>b</sup>	3.17±0.58 <sup>b</sup>	2.83±1.86

<sup>a</sup> One outlier has been removed (Grubbs test  $p < 0.05$ ).

<sup>b</sup> Significant difference ( $p < 0.05$ ) between QM1 and QM2.

<sup>c</sup> Significant difference ( $p < 0.05$ ) between QM1 and QM3.

<sup>d</sup> Significant difference ( $p < 0.05$ ) between QM2 and QM3.



Table 5. Reproducibility values for the SPME-GC-MS method expressed as the mean of the RSD%, calculated for each of the 15 analyzed samples (S1-S15). The concentration ranges (minimum and maximum values) and the mean RSD% values are also shown.

Compounds	Concentration range (mg/kg) in samples (S) - <i>Minimum (first row)/Maximum (second row)</i>															RSD% QM1 <sup>a</sup>	RSD% QM2 <sup>a</sup>	RSD% QM3 <sup>a</sup>
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15			
Octane	0.02 <sup>f</sup>	0.06	0.06	0.08	1.18	0.06	0.02 <sup>f</sup>	0.53	0.03	0.12	0.07	<0.01 <sup>ef</sup>	0.96	0.02 <sup>f</sup>	0.20	38.50 <sup>bc</sup>	68.01 <sup>bd</sup>	53.92 <sup>cd</sup>
	0.07	0.23	0.17	0.18	3.08	0.15	0.06	1.17	0.06	0.24	0.12	0.02 <sup>f</sup>	1.37	0.06	0.54			
Ethyl acetate	0.02 <sup>ef</sup>	0.11	<0.01 <sup>ef</sup>	0.65	0.62	0.82	0.51	0.16	0.09	0.70	0.29	0.03 <sup>f</sup>	0.14	0.11	0.16	28.17 <sup>bc</sup>	71.28 <sup>bd</sup>	51.93 <sup>cd</sup>
	0.04 <sup>f</sup>	0.22	0.01 <sup>f</sup>	0.92	0.72	1.65	0.94	0.28	0.17	0.92	0.53	0.06 <sup>f</sup>	0.37	0.19	0.34			
Ethanol	0.14	0.37	0.07 <sup>f</sup>	4.64	18.16	5.60	9.52	3.09	1.72	4.41	16.67	1.21	12.01	4.03	1.67	32.33 <sup>c</sup>	40.07 <sup>d</sup>	52.52 <sup>cd</sup>
	0.40	1.17	0.31	12.92	24.60	11.46	14.13	5.27	3.64	11.43	25.26	2.55	18.52	6.43	4.94			
Ethyl propanoate	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	0.02 <sup>f</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	38.96 <sup>c</sup>	48.81	69.72 <sup>c</sup>
	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.02 <sup>f</sup>	0.03 <sup>f</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.03 <sup>f</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>			
Hexanal	0.70	4.33	2.74	1.26	2.23	0.92	0.43	2.26	0.60	0.45	0.62	0.51	0.79	0.80	1.53	23.04 <sup>c</sup>	25.83 <sup>d</sup>	53.85 <sup>cd</sup>
	1.35	7.47	4.04	2.36	3.42	1.60	1.01	4.13	1.28	0.80	1.05	1.54	1.03	1.14	3.29			
3-Methyl-1-butanol	0.01 <sup>f</sup>	0.02 <sup>f</sup>	0.04	0.20	2.56	0.14	0.12	0.13	0.05	0.12	0.56	0.02 <sup>f</sup>	0.21	0.05	0.38	25.95 <sup>bc</sup>	64.65 <sup>bd</sup>	41.51 <sup>cd</sup>
	0.02 <sup>f</sup>	0.05	0.07	0.40	2.84	0.37	0.24	0.22	0.12	0.26	0.76	0.04	0.37	0.06	0.83			
<i>(E)</i> -2-Hexenal	9.02	11.01	0.84	6.48	2.20	5.21	3.72	3.32	3.05	1.90	1.42	9.38	2.09	22.73	18.16	19.55 <sup>bc</sup>	23.07 <sup>bd</sup>	46.91 <sup>cd</sup>
	16.98	16.83	1.53	9.34	4.65	7.71	6.01	4.81	4.74	2.82	2.57	15.93	3.31	43.32	23.85			
<i>(Z)</i> -3-Hexenyl acetate	<0.01 <sup>ef</sup>	0.23	1.56	0.63	0.09	0.20	2.59	1.16	2.78	1.15	0.17	0.62	0.20	1.78	0.09	19.18 <sup>bc</sup>	30.57 <sup>bd</sup>	62.04 <sup>cd</sup>
	0.01 <sup>f</sup>	0.39	2.70	0.77	1.08	3.07	4.56	1.80	5.19	1.55	0.27	0.90	0.29	3.03	0.21			
<i>(E)</i> -2-Heptenal	0.05	0.21	0.04 <sup>f</sup>	0.07	0.27	0.01 <sup>ef</sup>	0.02 <sup>f</sup>	0.16	0.02 <sup>f</sup>	0.02 <sup>f</sup>	0.02 <sup>f</sup>	0.01 <sup>ef</sup>	0.07	0.03 <sup>f</sup>	0.13	24.89 <sup>b</sup>	63.16 <sup>bd</sup>	36.16 <sup>d</sup>
	0.10	0.40	0.20	0.17	0.73	0.07	0.26	0.48	0.14	0.05	0.07	0.05	0.53	0.17	0.34			
6-Methyl-5-hepten-2-one	0.01 <sup>ef</sup>	0.28	0.16	0.02 <sup>f</sup>	0.24	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.24	0.02 <sup>f</sup>	0.02 <sup>f</sup>	0.09	<0.01 <sup>ef</sup>	0.26	0.01 <sup>ef</sup>	0.03 <sup>f</sup>	43.20 <sup>b</sup>	65.10 <sup>bd</sup>	61.64 <sup>d</sup>
	0.04 <sup>f</sup>	0.39	0.36	0.04 <sup>f</sup>	0.78	0.05 <sup>f</sup>	0.03 <sup>f</sup>	0.50	0.08	0.07	0.54	0.03 <sup>f</sup>	0.79	0.06	0.16			
1-Hexanol	0.14	0.27	1.33	0.61	1.65	1.72	1.10	0.68	0.36	1.01	0.21	0.42	1.84	0.80	1.03	13.26 <sup>bc</sup>	27.71 <sup>bd</sup>	59.96 <sup>cd</sup>
	0.30	0.89	2.72	0.82	2.01	2.46	1.54	0.69	0.53	1.24	0.32	0.94	4.15	1.54	1.21			
Nonanal	0.59	0.76	0.48	0.15	5.29	0.12	0.03 <sup>f</sup>	2.83	0.26	0.11	0.36	0.07 <sup>f</sup>	0.48	0.03 <sup>f</sup>	0.46	46.05	42.51	53.70
	1.54	4.80	1.75	1.53	8.65	1.17	0.94	5.41	0.83	1.57	0.94	0.35	1.36	0.58	2.52			
1-Octen-3-ol	0.01 <sup>f</sup>	0.03	0.02 <sup>f</sup>	0.01 <sup>f</sup>	0.06	0.01 <sup>f</sup>	<0.01 <sup>ef</sup>	0.03	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.02 <sup>f</sup>	<0.01 <sup>ef</sup>	0.02 <sup>f</sup>	<0.01 <sup>ef</sup>	0.02 <sup>f</sup>	31.48 <sup>c</sup>	38.87 <sup>d</sup>	64.07 <sup>cd</sup>
	0.01 <sup>f</sup>	0.05	0.03	0.02 <sup>f</sup>	0.18	0.01 <sup>f</sup>	0.01 <sup>f</sup>	0.05	0.01 <sup>f</sup>	0.01 <sup>f</sup>	0.03	<0.01 <sup>ef</sup>	0.04	0.01 <sup>f</sup>	0.07			
<i>(E,E)</i> -2,4-Hexadienal	0.06	0.05	0.03 <sup>f</sup>	0.02 <sup>f</sup>	0.01 <sup>f</sup>	0.03 <sup>f</sup>	0.03 <sup>f</sup>	0.02 <sup>f</sup>	0.06	0.12	0.01 <sup>f</sup>	0.14	0.04	0.27	0.08	63.46 <sup>c</sup>	69.01 <sup>d</sup>	105.47 <sup>cd</sup>
	0.58	0.62	0.14	0.31	0.53	0.51	0.25	0.20	0.83	0.46	0.06	1.16	0.12	1.20	1.03			
Acetic acid	0.19	1.20	0.30	2.46	3.94	9.63	0.79	0.89	0.37	3.99	0.62	0.27	0.38	0.42	0.26	17.47 <sup>c</sup>	22.81 <sup>d</sup>	71.83 <sup>cd</sup>
	0.45	3.67	0.62	6.52	8.95	25.06	1.98	2.12	0.62	12.75	1.68	0.58	0.84	0.75	0.72			
Propanoic acid	0.39	1.80	0.37	0.46	0.05	0.04	<0.01 <sup>ef</sup>	0.22	<0.01 <sup>ef</sup>	0.22	<0.01 <sup>ef</sup>	0.01 <sup>f</sup>	0.03	0.01 <sup>f</sup>	0.12	26.69 <sup>b</sup>	51.03 <sup>bd</sup>	25.19 <sup>d</sup>
	0.70	2.93	0.82	0.92	0.17	0.11	0.03	0.44	0.07	0.44	0.07	0.06	0.15	0.10	0.33			

Table cont.

<i>(E)</i> -2-Decenal	0.25 <sup>f</sup>	0.02 <sup>ef</sup>	0.04 <sup>ef</sup>	0.08 <sup>ef</sup>	0.49 <sup>f</sup>	0.10 <sup>ef</sup>	0.03 <sup>ef</sup>	0.30 <sup>f</sup>	0.04 <sup>ef</sup>	0.08 <sup>ef</sup>	0.04 <sup>ef</sup>	0.03 <sup>ef</sup>	0.01 <sup>ef</sup>	0.09 <sup>ef</sup>	0.20	36.65 <sup>c</sup>	54.33	61.52 <sup>c</sup>
	0.98	1.09	0.28 <sup>f</sup>	0.14 <sup>ef</sup>	3.57	0.13 <sup>ef</sup>	0.06 <sup>ef</sup>	2.26	0.06 <sup>ef</sup>	0.25 <sup>f</sup>	0.09 <sup>ef</sup>	0.03 <sup>ef</sup>	2.14	0.09 <sup>ef</sup>	1.18			
Pentanoic acid	0.85	0.22	0.02 <sup>ef</sup>	0.08 <sup>f</sup>	0.05 <sup>f</sup>	0.03 <sup>ef</sup>	0.01 <sup>ef</sup>	0.02 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.11 <sup>f</sup>	0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	<0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	0.01 <sup>ef</sup>	27.11 <sup>b</sup>	57.61 <sup>bd</sup>	25.51 <sup>d</sup>
	2.08	0.48	0.18	0.22	0.13 <sup>f</sup>	0.09 <sup>f</sup>	0.01 <sup>ef</sup>	0.09 <sup>f</sup>	0.04 <sup>ef</sup>	0.18	0.02 <sup>ef</sup>	0.05 <sup>f</sup>	0.06 <sup>f</sup>	0.02 <sup>ef</sup>	0.04 <sup>ef</sup>			

<sup>a</sup> Relative Standard Deviation (%) calculated as mean of RSD% for each compound among the involved labs by removing outliers.

<sup>b</sup> RSD% values obtained for QM1 and QM2 showed significant differences (p<0.05).

<sup>c</sup> RSD% values obtained for QM1 and QM3 showed significant differences (p<0.05).

<sup>d</sup> RSD% values obtained for QM2 and QM3 showed significant differences (p<0.05).

<sup>e</sup> Concentration is below the LOD (Table 7).

<sup>f</sup> Concentration is below the LOQ (Table 8).

Table 6. Mean and standard deviation values of recovery ( $R_{ap}$ ) calculated from the results of the labs involved using the three types of quantification methods (QMs). The recovery values are shown when the entire concentration range and low concentration range were applied in the calibration curves.

Volatile compounds	Whole concentration range (0.05-10.00/25.00 mg/kg) <sup>a</sup>			Low concentration range (0.05-2.5 mg/kg)		
	QM1	QM2	QM3	QM1	QM2	QM3
Octane	92±21	90±42	135±123	93±28	68±38 <sup>c</sup>	117±82
Ethyl acetate	99±22	94±46	118±79	74±10 <sup>c</sup>	54±31 <sup>c</sup>	94±28
Ethanol	104±67	131±80	138±104	71±39	71±45	108±85
Ethyl propanoate	101±18	96±44	128±87	86±12	64±37 <sup>c</sup>	103±39
Hexanal	106±11	150±67	266±221	119±42	114±53	188±142
3-Methyl-1-butanol	100±9	93±35	139±106	94±13	68±39	108±33
E-2-Hexenal	88±9	118±37	224±152	144±63	129±55	223±167
(Z)-3-Hexenyl acetate	88±5	121±54	248±180	159±82	139±60	267±227
(E)-2-Heptenal	102±25	92±21	157±96	152±56	92±23	180±139
6-Methyl-5-hepten-2-one	105±28	94±21	163±97	154±59	93±22	181±131
1-Hexanol	100±7	140±69	269±206	143±58	135±69	238±202
Nonanal	82±16	107±26	224±140	155±74	136±54	247±195
1-Octen-3-ol	86±8	121±53	252±175	166±80	147±63	283±246
(E,E)-2,4-Hexadienal	95±13	89±25	147±102	148±54	90±22	180±146
Acetic acid	84±26	105±11	208±146	125±72	115±72	157±104
Propanoic acid	94±25	88±37	119±44	111±26	76±36 <sup>c</sup>	115±26
(E)-2-Decenal	72±21	71±32	109±29 <sup>b</sup>	158±34 <sup>c</sup>	110±53 <sup>c</sup>	160±126
Pentanoic acid	99±16	92±22	184±172	184±87	114±47	223±250

<sup>a</sup> The highest concentration depended on the compound (see Table 2).

<sup>b</sup> Recovery values found for QM1 and QM3 showed significant differences ( $p < 0.05$ ). Non-significant differences were found between the recovery values of QM1 and QM2, and between QM2 and QM3 for all the compounds.

<sup>c</sup> Recovery values found for low concentration range and the whole concentration range showed significant differences ( $p < 0.05$ ).

Table 7. Mean values of LOD (mg/kg) for each VOC by applying four calculation methods; the ranges are also shown in parentheses for the first three methods.

<b>Volatile Compounds</b>	<b>Calculation Method 1</b>	<b>Calculation Method 2</b>	<b>Calculation Method 3</b>	<b>Calculation Method 4</b>
Octane	0.64 (0.18-0.89)	0.23 (0.06-0.31)	0.72 (0.06-1.27)	0.01
Ethyl acetate	0.44 (0.42-0.48)	0.19 (0.17-0.24)	0.43 (0.17-0.68)	0.03
Ethanol	1.29 (1.07-1.56)	0.45 (0.38-0.55)	0.54 (0.51-0.58)	0.03
Ethyl propanoate	0.25 (0.17-0.30)	0.10 (0.07-0.12)	0.22 (0.07-0.49)	0.02
Hexanal	1.69 (1.42-2.13)	0.53 (0.45-0.67)	1.43 (0.22-2.50)	0.02
3-Methyl-1-butanol	0.62 (0.38-0.84)	0.22 (0.13-0.29)	0.62 (0.29-0.90)	0.01
( <i>E</i> )-2-Hexenal	0.96 (0.07-1.64)	0.38 (0.03-0.64)	0.13 (0.05-0.27)	0.01
( <i>Z</i> )-3-Hexenyl acetate	1.00 (0.17-1.73)	0.39 (0.06-0.68)	0.15 (0.07-0.30)	0.01
( <i>E</i> )-2-Heptenal	0.92 (0.34-1.48)	0.32 (0.12-0.52)	0.16 (0.16-0.16)	0.02
6-Methyl-5-hepten-2-one	1.12 (0.72-1.55)	0.39 (0.25-0.54)	0.18 (0.10-0.24)	0.02
1-Hexanol	1.69 (0.73-2.22)	0.53 (0.23-0.70)	0.93 (0.47-1.18)	0.01
Nonanal	1.33 (0.21-2.09)	0.52 (0.08-0.83)	0.50 (0.10-0.76)	0.03
1-Octen-3-ol	1.58 (0.57-2.47)	0.53 (0.19-0.83)	0.52 (0.25-0.69)	0.01
( <i>E,E</i> )-2,4-Hexadienal	0.87 (0.34-1.73)	0.31 (0.12-0.61)	0.12 (0.08-0.17)	0.01
Acetic acid	1.83 (0.85-2.63)	0.59 (0.28-0.85)	0.92 (0.59-1.18)	0.01
Propanoic acid	0.58 (0.27-1.18)	0.20 (0.10-0.41)	0.36 (0.11-0.51)	0.01
( <i>E</i> )-2-Decenal	1.60 (1.19-2.40)	0.56 (0.42-0.84)	0.57 (0.41-0.68)	0.18
Pentanoic acid	0.98 (0.31-1.42)	0.34 (0.11-0.50)	0.19 (0.14-0.25)	0.05

Note: n/a: not available as not detectable.

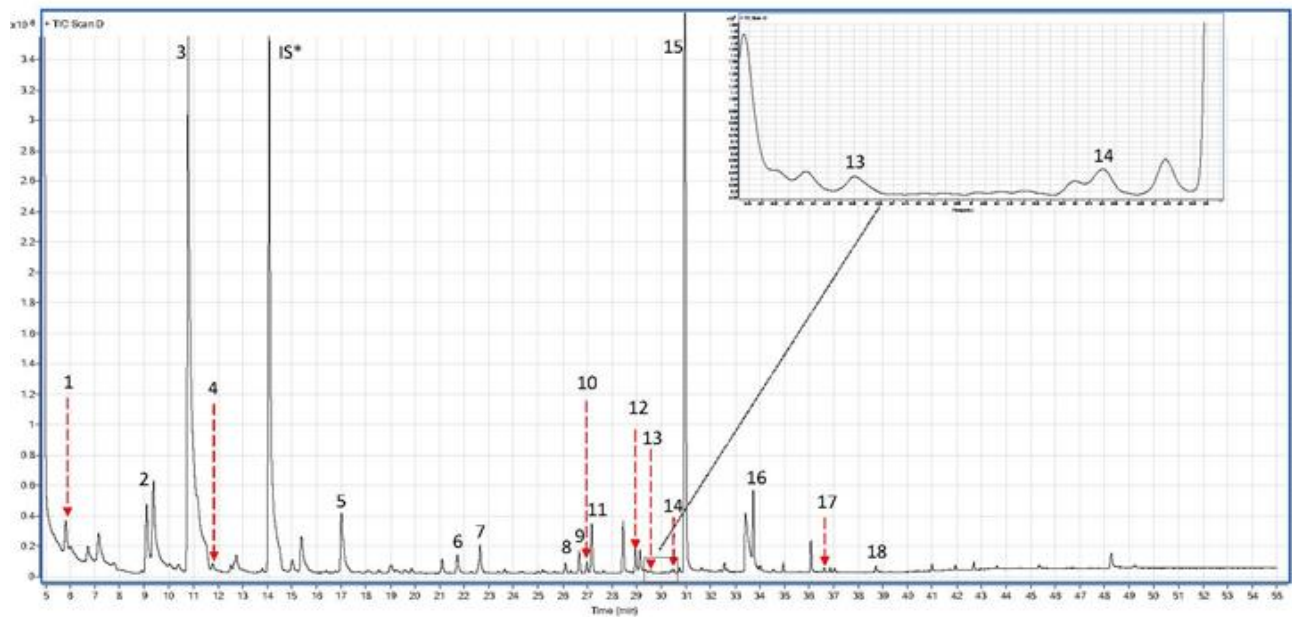
Table 8. Mean values of the LOQ (mg/kg) for each volatile compound by applying four calculation methods; the ranges are shown in parentheses for the first three methods.

Volatile Compounds	Calculation Method	Calculation Method	Calculation Method	Calculation Method
	1	2	3	4
Octane	1.95 (0.56-2.69)	0.68 (0.20-0.95)	2.18 (0.19-3.85)	0.03
Ethyl acetate	1.35 (1.26-1.45)	0.58 (0.50-0.73)	1.31 (0.52-2.07)	0.08
Ethanol	3.91 (3.24-4.72)	1.38 (1.14-1.65)	1.64 (1.54-1.74)	0.09
Ethyl propanoate	0.74 (0.52-0.92)	0.30 (0.21-0.37)	0.67 (0.20-1.47)	0.07
Hexanal	5.11 (4.30-6.46)	1.62 (1.37-2.04)	4.34 (0.68-7.58)	0.07
3-Methyl-1-butanol	1.89 (1.14-2.55)	0.66 (0.40-0.89)	1.89 (0.87-2.72)	0.04
( <i>E</i> )-2-Hexenal	2.90 (0.22-4.97)	1.14 (0.09-1.95)	0.38 (0.15-0.82)	0.03
( <i>Z</i> )-3-Hexenyl acetate	3.03 (0.50-5.24)	1.20 (0.19-2.06)	0.46 (0.21-0.91)	0.02
( <i>E</i> )-2-Heptenal	2.79 (1.04-4.48)	0.97 (0.36-1.57)	0.48 (0.47-0.49)	0.05
6-Methyl-5-hepten-2-one	3.41 (2.19-4.70)	1.19 (0.77-1.64)	0.55 (0.30-0.74)	0.06
1-Hexanol	5.11 (2.23-6.73)	1.62 (0.70-2.13)	2.82 (1.42-3.59)	0.01
Nonanal	4.02 (0.65-6.33)	1.58 (0.25-2.50)	1.52 (0.30-2.31)	0.09
1-Octen-3-ol	4.80 (1.73-7.47)	1.61 (0.58-2.52)	1.57 (0.76-2.09)	0.03
( <i>E,E</i> )-2,4-Hexadienal	2.65 (1.03-5.25)	0.93 (0.36-1.84)	0.37 (0.25-0.51)	0.04
Acetic acid	5.53 (2.58-7.98)	1.79 (0.84-2.58)	2.79 (1.78-3.57)	0.04
Propanoic acid	1.75 (0.82-3.57)	0.61 (0.29-1.25)	1.11 (0.34-1.54)	0.02
( <i>E</i> )-2-Decenal	4.85 (3.62-7.28)	1.69 (1.27-2.54)	1.72 (1.24-2.07)	0.53
Pentanoic acid	2.96 (0.94-4.29)	1.03 (0.33-1.50)	0.59 (0.43-0.76)	0.15

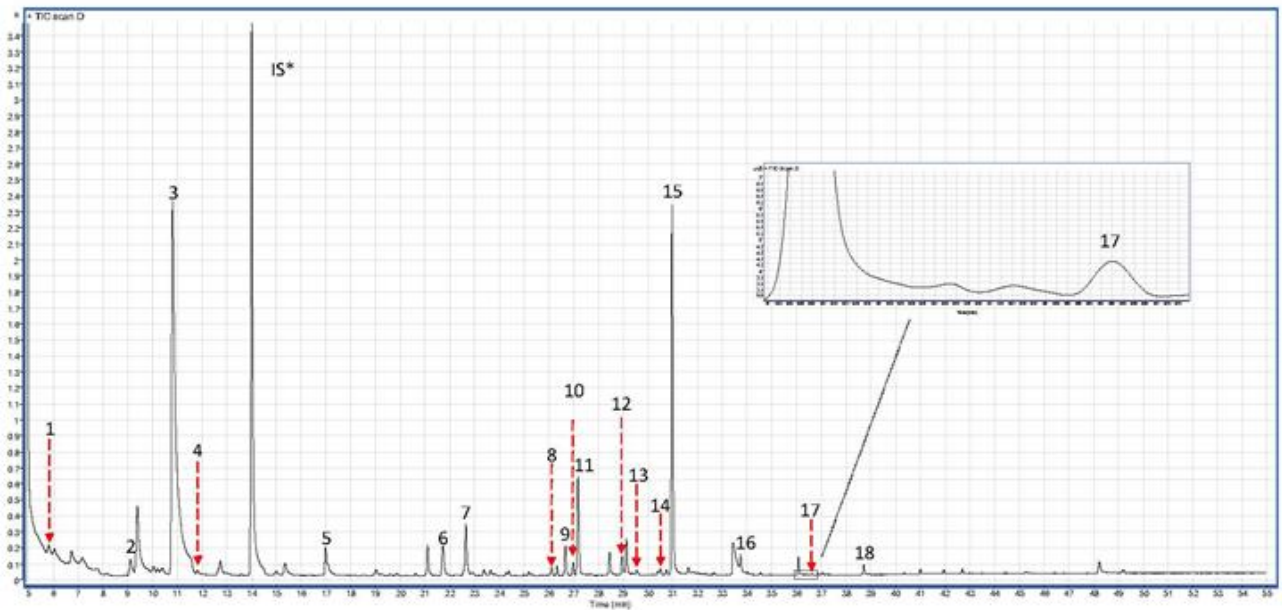
Note: n/a: not available as not detectable.

**FIGURE 1**

### Lampante olive oil

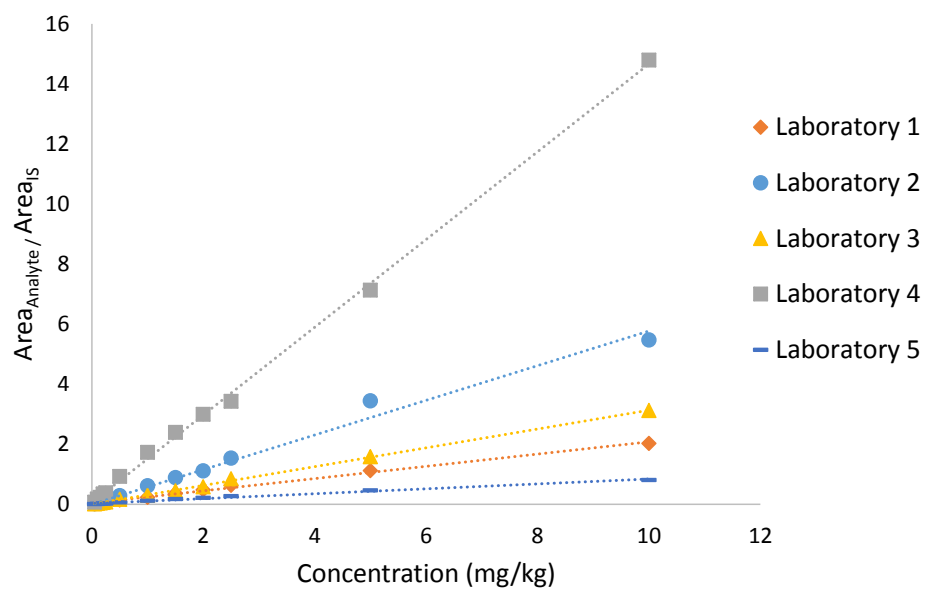


### Virgin olive oil



**Figure 1.** Chromatogram of volatile compounds of a lampante olive oil and a virgin olive analyzed by SPME-GC-MS. The correspondence of the codes with the volatile compounds is shown in Table 1.

**FIGURE 2**



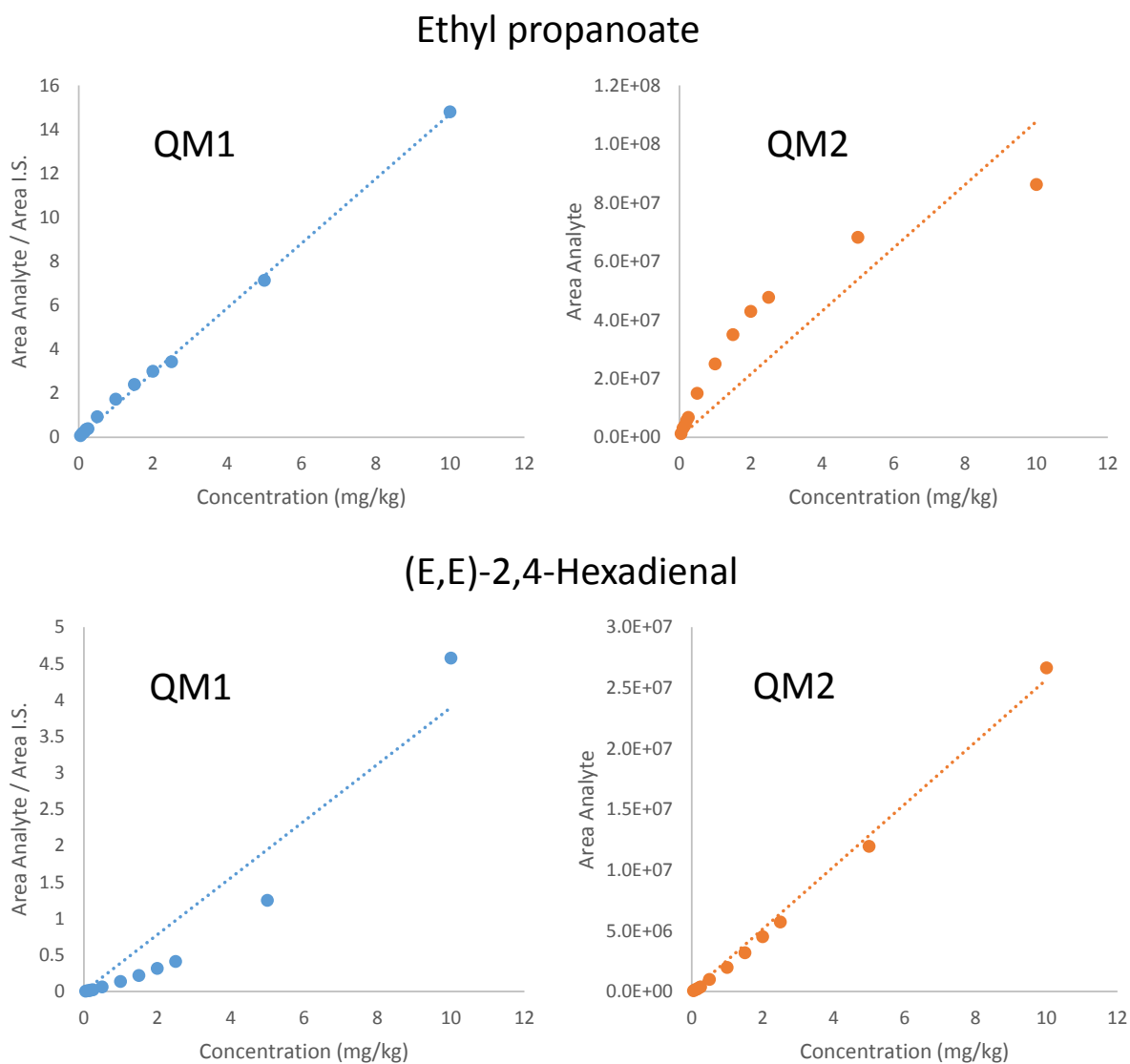
**Figure 2.** Calibration curves of ethyl propanoate for quantification method 2 (QM2).

## SUPPLEMENTARY INFORMATION

### **Collaborative validation trial of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils**

Ramón Aparicio-Ruiz<sup>1</sup>, Clemente Ortiz Romero<sup>1</sup>, Diego L. García González<sup>1</sup>, Maurizio Servili<sup>2</sup>,  
Roberto Selvaggini<sup>2</sup>, Florence Lacoste<sup>3</sup>, Julien Escobessa<sup>3</sup>, Stefania Vichi<sup>4</sup>, Beatriz Quintanilla-  
Casas<sup>4</sup>, Pierre Alain Golay<sup>5</sup>, Paolo Lucci<sup>6</sup>, Erica Moret<sup>6</sup>, Enrico Valli<sup>7\*</sup>, Alessandra Bendini<sup>7</sup>,  
Tullia Gallina Toschi<sup>7</sup>





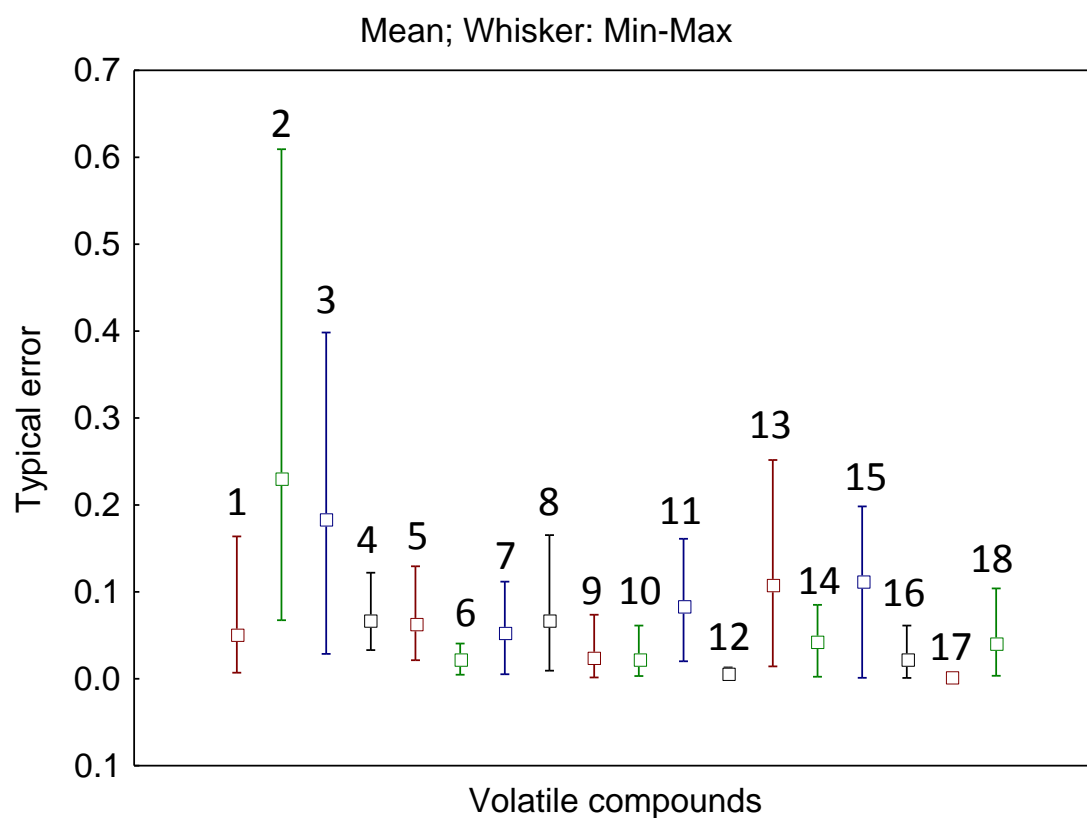
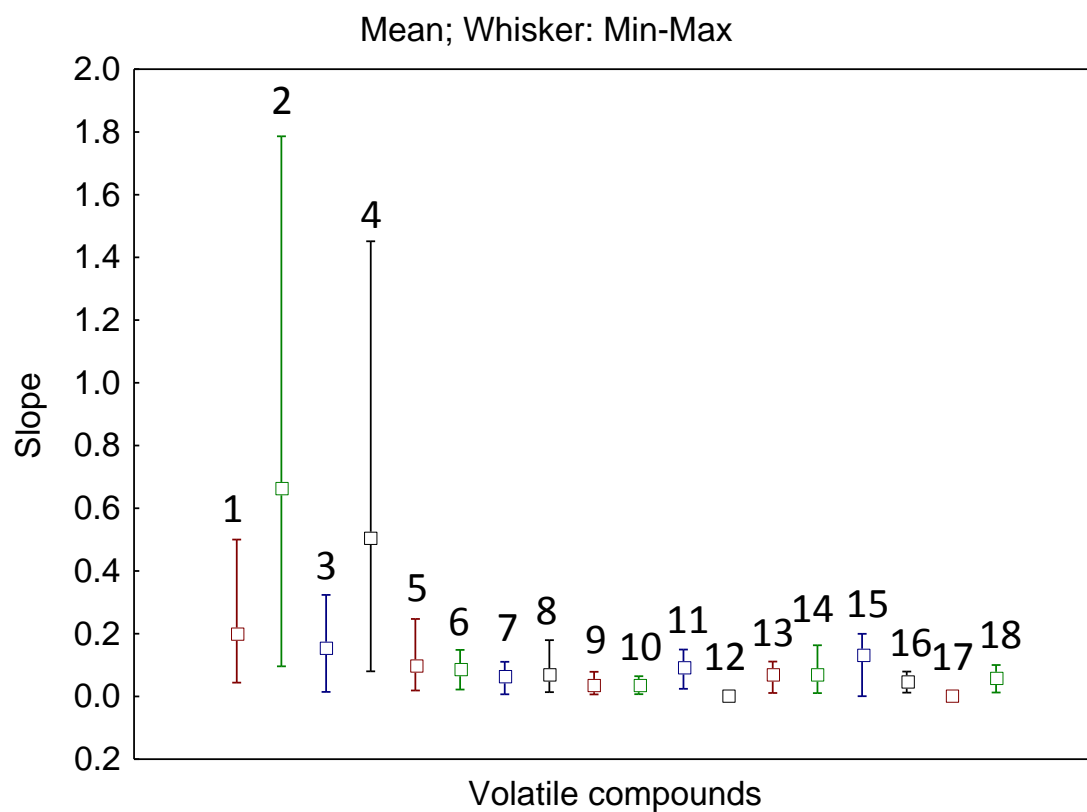
**Figure S1.** Calibration curves of ethyl propanoate and (E,E)-2,4,hexadienal built in quantification methods 1 and 2 (QM1, QM2).

**Table S2.** Slope and typical error (mean±standard deviation) of the regression equation built for the calibration curves (QM1).

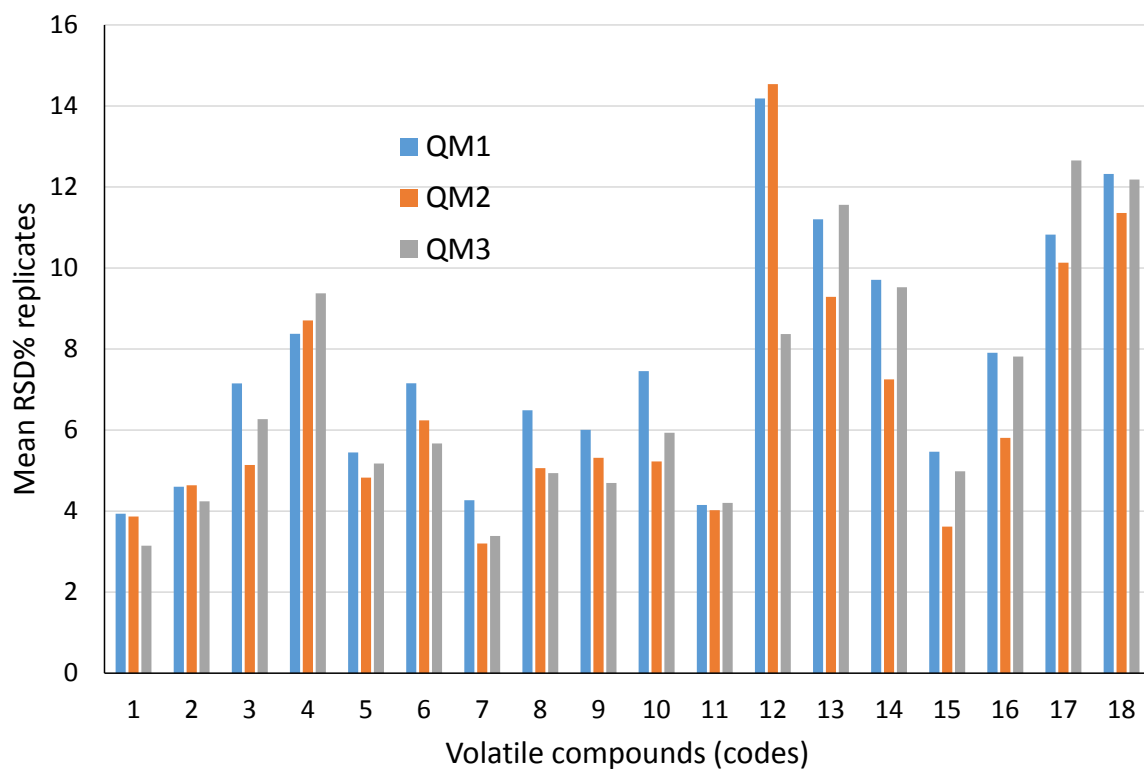
<b>Volatile compounds</b>	<b>Slope (Mean±SD)</b>	<b>Typical error (Mean±SD)</b>
Octane	0.201±0.186	0.052±0.066
Ethyl acetate	0.666±0.655	0.231±0.238
Ethanol	0.159±0.136	0.184±0.163
Ethyl propanoate	0.508±0.552	0.067±0.035
Hexanal	0.099±0.089	0.064±0.045
3-Methyl-1-butanol	0.091±0.057	0.022±0.016
( <i>E</i> )-2-Hexenal	0.064±0.047	0.053±0.043
( <i>Z</i> )-3-Hexenyl acetate	0.072±0.064	0.068±0.070
( <i>E</i> )-2-Heptenal	0.037±0.030	0.024±0.030
6-Methyl-5-hepten-2-one	0.036±0.026	0.023±0.024
1-Hexanol	0.092±0.046	0.085±0.069
Nonanal	0.004±0.003	0.006±0.005
1-Octen-3-ol	0.073±0.044	0.108±0.104
( <i>E,E</i> )-2,4-Hexadienal	0.073±0.061	0.044±0.038
Acetic acid	0.135±0.080	0.112±0.079
Propanoic acid	0.052±0.028	0.022±0.024
( <i>E</i> )-2-Decenal	0.002±0.002	0.002±0.003
Pentanoic acid	0.058±0.034	0.041±0.041

**Table S3.** Linearity in the low concentration range of the calibration curve (0.05-0.25 mg/kg) (QM1).

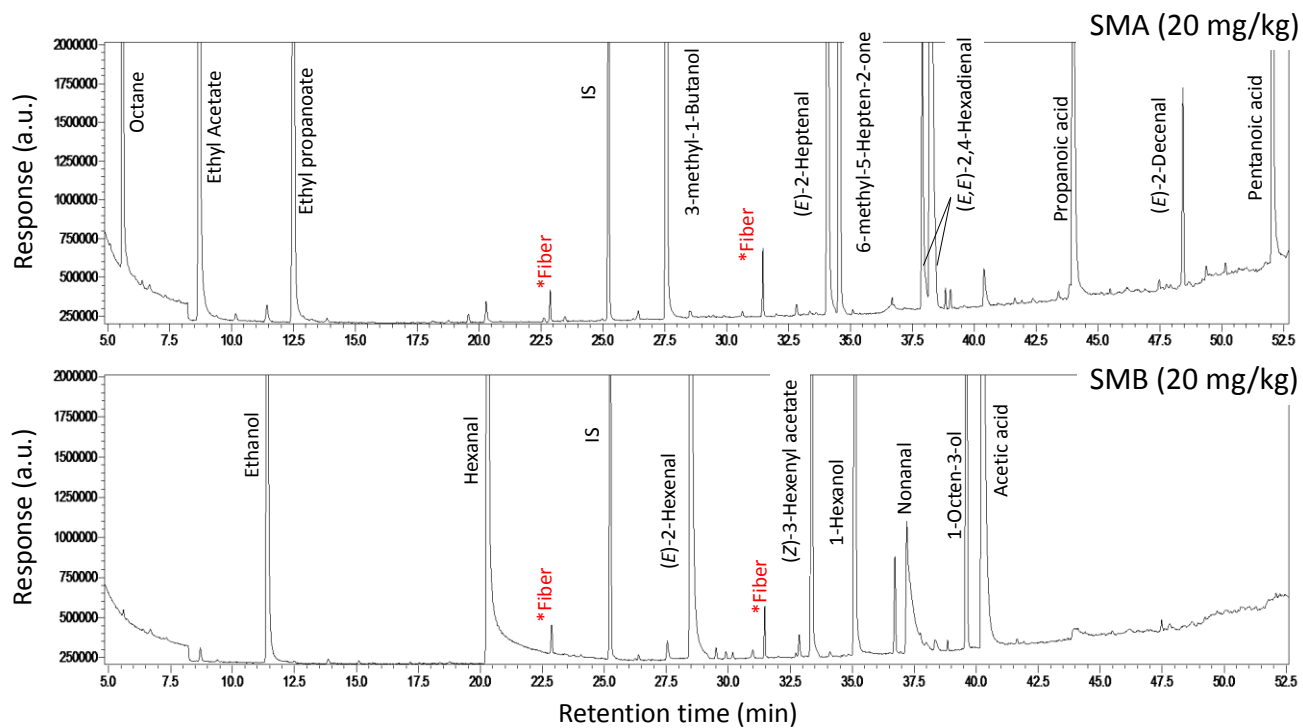
<b>Volatile compound</b>	<b>R<sup>2</sup></b>	<b>Slope</b>	<b>Intercept</b>
Octane	0.972±0.030	0.152±0.101	0.002±0.003
Ethyl acetate	0.978±0.026	0.959±0.979	0.004±0.007
Ethanol	0.963±0.051	0.246±0.240	-0.003±0.014
Ethyl propanoate	0.975±0.034	0.532±0.543	0.002±0.004
Hexanal	0.964±0.034	0.093±0.075	0.001±0.002
3-Methyl-1-butanol	0.969±0.030	0.112±0.076	-0.001±0.001
( <i>E</i> )-2-Hexenal	0.941±0.107	0.044±0.040	-0.001±0.001
( <i>Z</i> )-3-Hexenyl acetate	0.987±0.009	0.055±0.063	-0.001±0.001
( <i>E</i> )-2-Heptenal	0.984±0.021	0.017±0.009	0.000±0.000
6-Methyl-5-hepten-2-one	0.980±0.018	0.019±0.011	0.000±0.000
1-Hexanol	0.979±0.028	0.065±0.042	0.000±0.000
Nonanal	0.613±0.436	0.001±0.000	0.001±0.001
1-Octen-3-ol	0.976±0.020	0.039±0.028	-0.033±0.058
( <i>E,E</i> )-2,4-Hexadienal	0.986±0.019	0.051±0.034	-0.001±0.001
Acetic acid	0.977±0.019	0.132±0.089	0.014±0.018
Propanoic acid	0.975±0.021	0.044±0.031	0.000±0.001
( <i>E</i> )-2-Decenal	0.629±0.431	0.000±0.000	0.000±0.000
Pentanoic acid	0.908±0.109	0.020±0.014	0.001±0.001



**Figure S2.** Box and whisker plots of the slope and typical error (mean±standard deviation) of the regression equation built for the calibration curves (QM1). The volatile compound codes correspond to Table 1.



**Figure S3.** Mean relative standard deviation (RSD%) computed from the duplicates of the 15 samples analyzed by the 5 laboratories. The volatile compound codes correspond to Table 1.



**Figure S4.** Chromatograms of the standard mixtures SMA and SMB built for calibration (calibration point 20 mg/kg). Note: \*Compounds deriving from the SPME divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber.

**Collaborative peer validation of a harmonized SPME-GC-MS method  
for analysis of selected volatile compounds in virgin olive oils**

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*Highlights:*

- A SPME-GC-MS based protocol with 3 possible quantification methods was developed.
- A peer-interlab study was carried out (5 labs, 18 volatiles, 15 virgin olive oils).
- Results were compared with a similar study carried with FID detector.
- Linearity, recovery, precision and repeatability were satisfactory.
- Three compounds showed reproducibility RSD >40%, partially due to high LOQs.

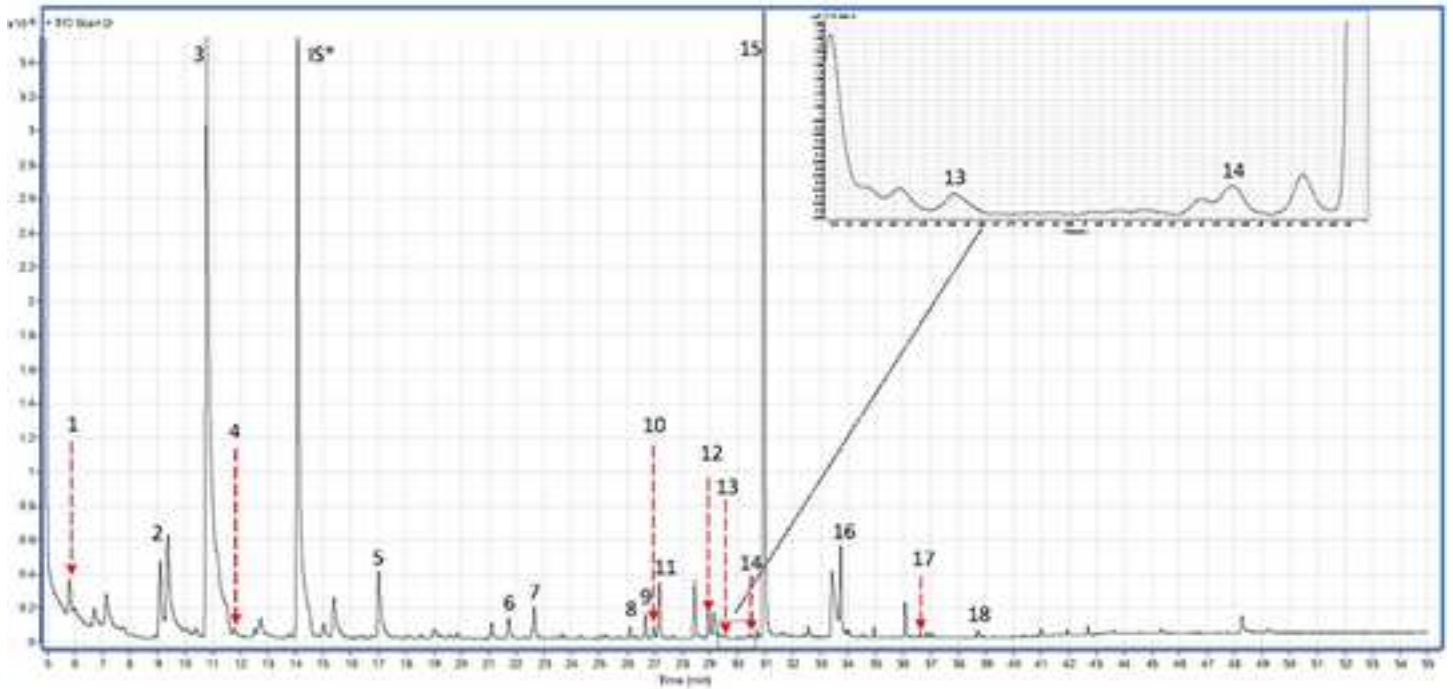
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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

### Lampante olive oil



### Virgin olive oil

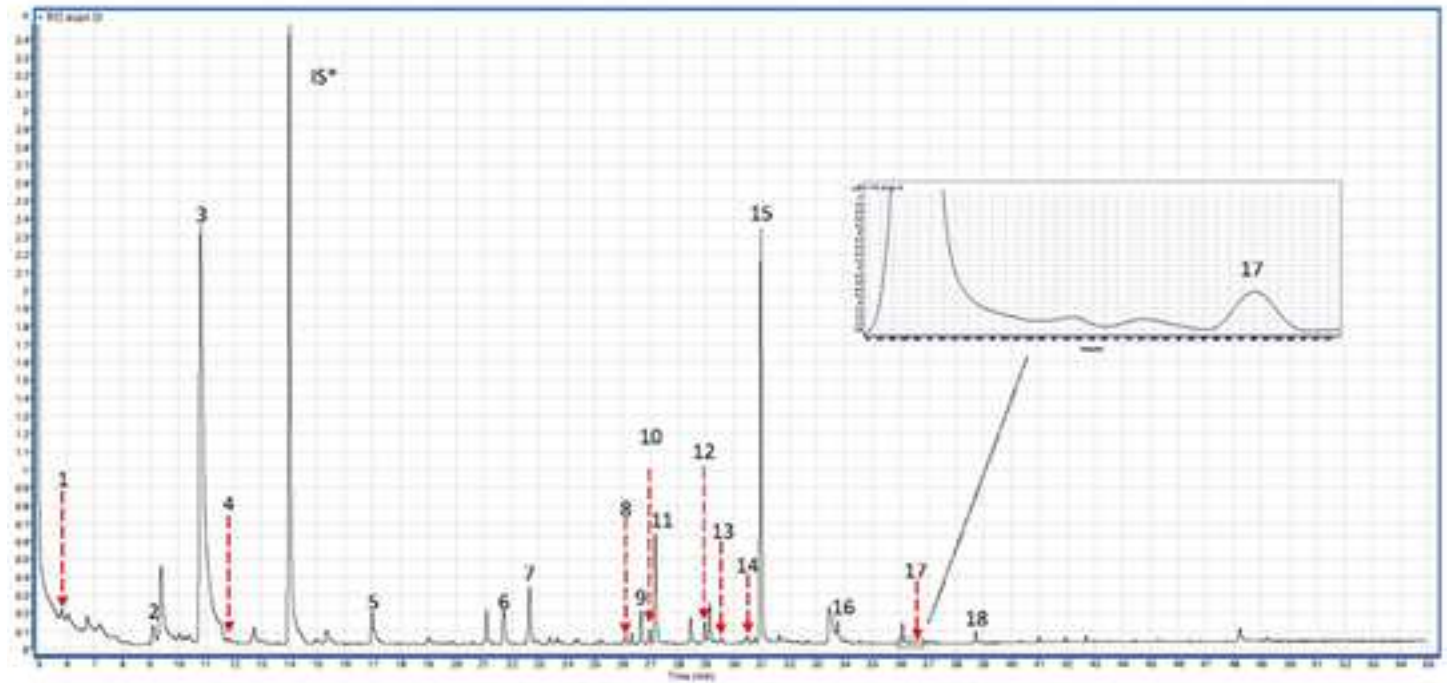
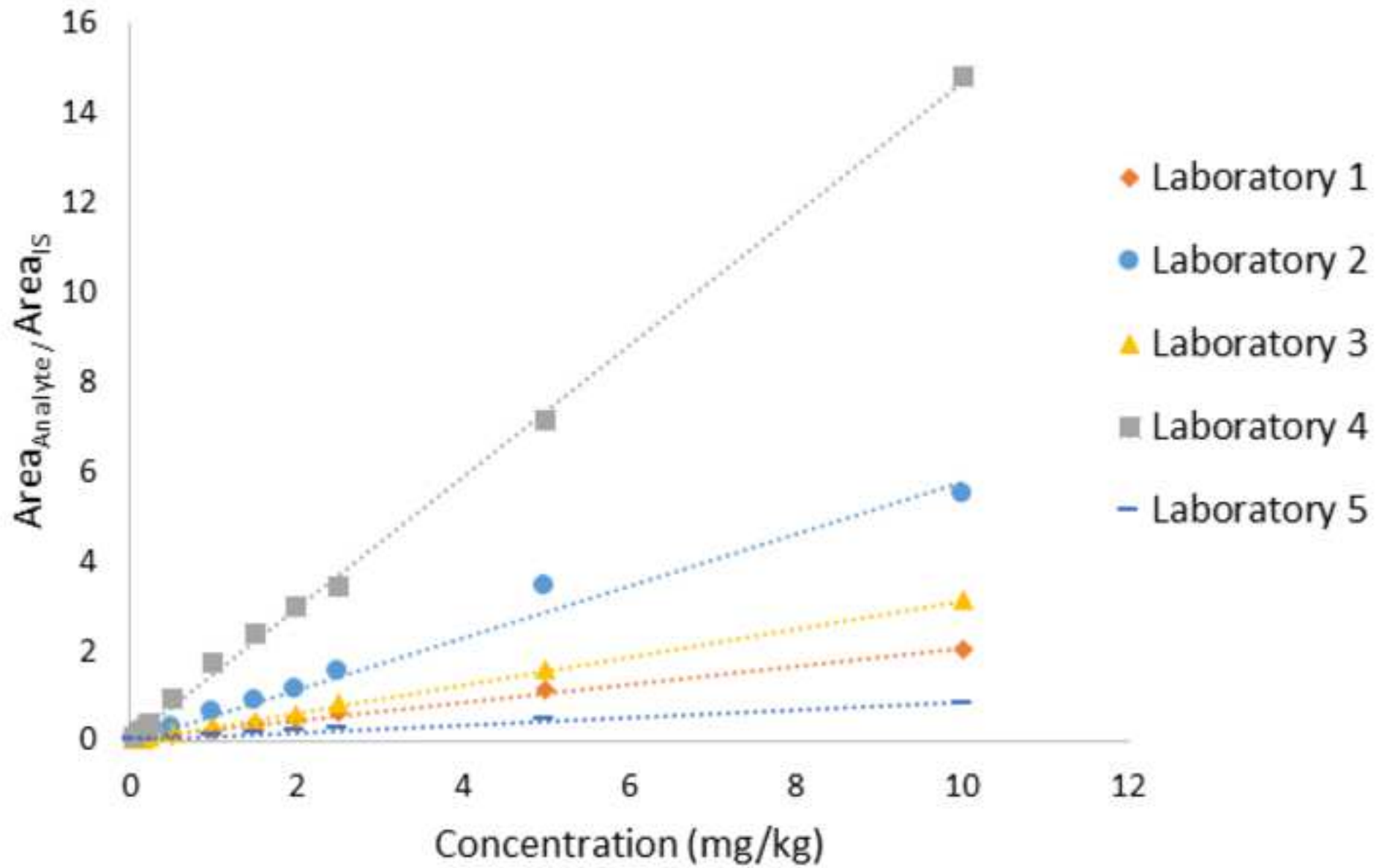




Figure 2





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