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1 **Development and validation of an LC-FTMS method for quantifying natural sweeteners in**
2 **wine**

3

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21

22 **Abstract**

23 The quality of a wine largely depends on the balance between its sourness, bitterness and
24 sweetness. Recently, *epi*-dihydrophaseic acid-3'-*O*- β -glucopyranoside (*epi*-DPA-G) and astilbin,
25 two molecules obtained from grapes, have been shown to contribute notably to the sweet taste of
26 dry wines. To study the parameters likely to affect their concentration, a new method was
27 developed and optimized by LC-FTMS. Three gradients and five C18 columns were tested. Good
28 results in terms of linearity ($r^2 > 0.9980$), repeatability ($RSD \leq 3\%$), recovery ($\geq 89\%$) and LOQ
29 ($\leq 20 \mu\text{g}\cdot\text{L}^{-1}$) were obtained. The method was used to screen *epi*-DPA-G and astilbin in red wines
30 of several vintages over one century. Both compounds were detected in all wines at
31 concentrations varying from 1.2 to 14.7 mg/L for *epi*-DPA-G and from 0.5 to 42.6 mg/L for
32 astilbin. Therefore, this new method can be used to quantify *epi*-DPA-G and astilbin reliably in
33 wine.

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36 **Keywords:** Orbitrap, method validation, wine, *epi*-DPA-G, astilbin, sweetness

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41 **1. Introduction**

42 Wine is an alcoholic beverage that has been produced and praised for thousands of years on
43 almost every continent and is considered to be a combination of art and science (Haseeb, Santi,
44 Liprandi, & Baranchuk, 2019). At the molecular level, it consists of a matrix containing
45 thousands of different molecules in several compound classes, all suspended in a hydro-ethanolic
46 medium at varying concentrations (De Revel et al., 2017; Lorrain, Ky, Pechamat, & Teissedre,
47 2013; Markoski, Garavaglia, Oliveira, Olivaes, & Marcadenti, 2016). These compounds can be
48 extracted from grapes, synthesized by microorganisms, released from oak wood during
49 winemaking, and even formed during bottle aging (Ribereau-Gayon, Dubourdieu, Doneche, &
50 Lonvaud, 2017). Therefore, the taste, aroma and composition of wine can be understood by
51 studying grapes and wine chemistry. Scientific breakthroughs in enology have led to practical
52 benefits and have significantly contributed to better monitoring of winemaking.

53 The sensory properties of a wine are major elements that determine its success among consumers
54 and thus its value (Coste, Sousa, & Malfeito-Ferreira, 2018; Francis & Williamson, 2015;
55 Loureiro, Brasil, & Malfeito-Ferreira, 2016). For example, consumers' spontaneous appetite for
56 the sweet taste in wine is well known (MadalenaSena-Esteves, Mota, & Malfeito-Ferreira, 2018).
57 Wine sweetness is important because it contributes to the gustatory balance by reducing the
58 acidity, bitterness and astringency generated by organic acids and polyphenols (Hufnagel &
59 Hofmann, 2008). These sensory interactions occur even in dry wines, i.e. wines with sugar
60 contents far lower than their detection threshold. In dry wines, it has been shown that sweetness
61 increases with the contact of yeast lees (Marchal, Marullo, Moine, & Dubourdieu, 2011) and
62 during oak aging (Marchal, Pons, Lavigne, & Dubourdieu, 2013). These phenomena have been
63 explained at the molecular level by demonstrating respectively the contribution of the protein

64 Hsp12 (Marchal, Marullo, et al., 2011) and by identifying sweet oak triterpenoids called
65 quercotriterpenosides (QTT) (Marchal, Waffo-Teguo, Génin, Mérillon, & Dubourdieu, 2011).
66 Recently, several sweet-tasting compounds from grapes, and especially seeds, have been
67 identified in dry wines (Crétin, Waffo-Teguo, Dubourdieu, & Marchal, 2019, p.), especially *epi*-
68 dihydrophaseic acid-3'-*O*- β -glucopyranoside, *epi*-DPA-G and astilbin (Crétin, 2016; Crétin et al.,
69 2019). Astilbin is a flavonoid, while *epi*-DPA-G is a glucosylated abscisic acid derivate (Del
70 Refugio Ramos et al., 2004). The identification of these compounds in wine has opened
71 promising perspectives to better understand the molecular determinism of wine taste and to
72 monitor its balance. For this reason, a reliable quantitation method is needed to establish the
73 influence of viticultural and enological factors on *epi*-DPA-G and astilbin concentrations in wine.
74 As wine is a complex matrix containing thousands of compounds and because some of them can
75 have a significant sensory impact even at trace level, elucidating the molecular determinants of
76 wine taste requires overcoming a dual challenge (L. Waterhouse, L. Sacks, & W. Jefferey, 2016).
77 First, the use of analytical assays must allow the resolute separation of wine components. For
78 instance, high performance liquid chromatography (HPLC), gas chromatography (GC) and/or
79 capillary electrophoresis (CE) have already been used (Acunha, Simó, Ibáñez, Gallardo, &
80 Cifuentes, 2016; V. Esteves, Lima, Lima, & Duarte, 2004; Malec et al., 2017; Pinto et al., 2019).
81 Sensitive and selective spectroscopic techniques such as mass spectrometry (MS) or nuclear
82 magnetic resonance must be used to identify active compounds (Pinto et al., 2019).
83 In particular, liquid chromatography (LC) coupled to Fourier transform mass spectrometry
84 (FTMS) with an Orbitrap analyzer has been used for a decade to analyze a broad range of
85 compounds in various foods and beverages. The method is very sensitive and covers a wide
86 dynamic range (Hogenboom, van Leerdam, & de Voogt, 2009). In combination with a high mass

87 resolution and accuracy in mass measurement, it is particularly powerful for applications
88 involving structural identification, qualitative screening and quantification.

89 In this work, a new LC-FTMS method was developed to quantify *epi*-DPA-G and astilbin in
90 wine. Three different gradients were tested on five different C18 columns, and performance
91 parameters such as linearity, inter- and intra-day repeatability of retention time and peak area
92 (RSD_{tr} and RSD_A), sensitivity (LOD, LOQ) and recovery were evaluated. The validated method
93 was successfully applied to quantify these sweet molecules in several commercial wines. Sixteen
94 vintages of a famous Burgundy estate were analyzed to assess the presence of the two sweet-
95 tasting compounds in old wines up to one century old. These results established the first
96 quantitative data of *epi*-DPA-G in wine.

97

98 **2. Materials and methods**

99 **2.1. Chemicals and commercial wines**

100 Ultrapure water (Milli-Q purification system, Millipore, France) and HPLC grade methanol
101 (VWR International, Pessac, France) were used for sample preparation. Acetonitrile, water LC-
102 MS grade and formic acid used for mass spectrometry analysis were purchased from Fisher
103 Chemical (Illkirch, France). Sixty-eight commercial red wines from 1918 to 2017 obtained from
104 different varieties and areas were analyzed to assess the presence of astilbin and *epi*-DPA-G
105 (**Table 1**).

106 **2.2. Sample preparation**

107 Stock solutions of *epi*-DPA-G and astilbin (chromatographically pure at 96 %), isolated in a
108 previous study (Crétin et al., 2019) were prepared in methanol at 1 mg/mL and stored at 4 °C.

109 Each sample of commercial wine was diluted to 1/3 in pure water and 0.45 μm -filtered before
110 injection in LC-FTMS in order to prevent column saturation and to decrease the ethanol level
111 likely to affect the chromatographic separation.

112 **2.3. Instrumentation and operating conditions**

113 The LC-FTMS platform consisted of an HTC PAL autosampler (CTC Analytics AG, Zwingen,
114 Switzerland), an Accela U-HPLC system with quaternary pumps and an Exactive Orbitrap mass
115 spectrometer equipped with a heated electrospray ionization (HESI I) probe (both from Thermo
116 Fisher Scientific, Les Ulis, France). Different C18 columns were tested in this study: Hypersil
117 Gold (2.1 mm x 100 mm, 1.9 μm), Synchronis™ (100 mm x 2.1 mm, 1.7 μm) from Thermo
118 Fisher Scientific, High Silica Strength (HSST3; 100 mm x 2.1 mm, 1.8 μm), Bridged
119 Ethylsiloxane/silica Hybrid (BEH; 100 mm x 2.1 mm, 1.7 μm) from Waters and Kinetex (100
120 mm x 2.1 mm, 1.7 μm) from Phenomenex. All the columns were protected by a guard column.
121 Five μL of each sample were injected in a full injection mode. When using HSST3, BEH and
122 Synchronis, the gradient ran at a constant flow rate of 400 $\mu\text{L}/\text{min}$ while with Hypersil and
123 Kinetex the flow rate was set at 600 $\mu\text{L}/\text{min}$. The eluents were (A) 0.1 % formic acid in water
124 and (B) 0.1 % formic acid in acetonitrile. Three different gradients were tested. Gradient **I**
125 consisted of 5 % (B) at 0 min; 5 % at 1 min; 30 % at 5.30 min; 98 % at 6.20 min ; 98 % at 6.45
126 min ; 5 % at 7.80 min and 5 % at 9 min. Gradient **II** consisted of 2 % (B) at 0 min; 2 % at 1 min;
127 25 % at 5 min; 98 % at 5.30 min ; 98 % at 6.30 min ; 2 % at 6.45 min and 2 % at 9 min. Gradient
128 **III** consisted of 10 % (B) at 0 min; 15 % at 1 min; 25 % at 3 min; 80 % at 5.5 min; 90 % at 7.5
129 min and 10 % at 9 and 10 min.

130 Mass acquisitions were performed in negative Fourier Transform Mass Spectrometry (FTMS)
131 ionization mode at a resolution of 10 000 ($m/\Delta m$, fwhm at 200 Th). The mass analyzer was

132 calibrated each week using Pierce® ESI Negative Ion Calibration Solution (Thermo Fisher
133 Scientific). The sheath and auxiliary gas flows (both nitrogen) were optimized at 80 and 15
134 arbitrary units, respectively. The HESI probe and capillary temperatures were 320 and 350 °C,
135 respectively. The electrospray voltage was set at – 3.5 kV, the capillary voltage to – 25 V, the
136 tube lens voltage offset to – 120 V and the skimmer voltage to – 20 V. Mass spectra were
137 recorded from 160 to 2000 Th, with an AGC value of 10⁶. All data were processed using the
138 Qualbrowser and Quanbrowser applications of Xcalibur version 2.1 (Thermo Fischer Scientific).

139 **2.4. Method validation**

140 To choose the best chromatographic conditions and to validate the LC-FTMS method, the
141 following parameters were evaluated on the five columns in a PO1988.

142 **2.4.1. Calibration curve and linearity**

143 Calibration curves were designed by plotting the *epi*-DPA-G and astilbin areas obtained (y_i)
144 against the nominal concentration of each calibration standard (x_i). Different concentrations were
145 tested; 0.02, 0.05, 0.08, 0.2, 0.5, 0.8, 1, 5, 8 and 10 mg/L. Linear regression was performed and
146 the correlation coefficient (r^2), slope (a) and intercept (b) were determined.

147 **2.4.2. Intra- and inter-day precision (RSD)**

148 Intra- and inter-assay accuracy and precision were evaluated for *epi*-DPA-G and astilbin by terms
149 of relative standard deviation (RSD) on retention time (t_r) and peak area (A) with five replicates
150 (n=5) at seven different levels on a single assay and five assays on three non-consecutive days.

151 **2.4.3. Limits of detection (LOD) and quantification (LOQ)**

152 Due to high mass accuracy, the noise level in the Orbitrap mass spectrometer, especially at $m/z >$
 153 200, is virtually absent. Consequently, a standard signal-to-noise approach to determine LOQ and
 154 LOD is not relevant (De Paepe et al., 2013). Therefore, LOD and LOQ were estimated using an
 155 approach of linearity recommended by the International Organization of Vine and Wine
 156 (www.oiv.int/public/medias/2754/oiv-ma-as1-12fr.pdf). It uses the data obtained from the
 157 linearity or calibration curve such as the slope a and the standard deviation of the intercept of the
 158 regression S_b . Therefore, S_b corresponds to:

$$159 \quad S_b = S_{res} \sqrt{\left(\frac{1}{np} + \frac{Mx^2}{\sum p(xi-Mx^2)}\right)} \quad (1)$$

160 And S_{res} to:

$$S_{res} = \sqrt{\frac{\sum_{i=1}^n \sum_{j=1}^p (y_{i,j} - \hat{y}_{i,j})^2}{pn-2}} \quad (2)$$

161 Where n =number of injections; p = number of repetitions;

162 Mx^2 = average of x values and \hat{y}_j = theoretical value obtained from the calibration curve

163 Using these parameters, LOD corresponds to $(3 \times S_b)/a$ and LOQ to $(10 \times S_b)/a$.

164 2.5. Study of commercial wines

165 The appropriate chromatographic conditions were used to screen and quantify the *epi*-DPA-G
 166 and astilbin present in different vintages of red wine (**Table 1**).

167 2.6. Recovery

168 Recovery was analyzed with three different samples of wines (PO1999b, SES2001 and
 169 VPCR1992) spiked with three concentrations of *epi*-DPA-G and astilbin (100 μ g/L, 500 μ g/L
 170 and 1 mg/L; $n=3$). The concentration determined by means of the calibration model was

171 compared to the real concentration of the standard by calculating the recovery rate ((determined
172 concentration/real concentration) × 100) (Thompson, Ellison, & Wood, 2002).

173 **3. Results and discussion**

174 **3.1. Optimization of chromatographic conditions**

175 A taste-guided methodology was previously developed to isolate sweet compounds from wine.
176 Their chemical structure was determined by HRMS and NMR (Crétin et al., 2019). This latter
177 study has led to significant advances in knowledge of wine flavor by revealing sweet compounds
178 obtained from grapes, especially *epi*-DPA-G and astilbin. In addition, a method for quantitating
179 their presence and concentrations in various commercial wines was developed in the present
180 work. Given the chemical complexity of the wine matrix, it was appropriate to use LC-FTMS.
181 Previously, Huang and Liaw (Huang & Liaw, 2017) developed a UPLC-DAD-MS method to
182 analyze astilbin in *Hypericum formosanum* using the XBridge C18 column and a mobile phase
183 composed of 0.1 % formic acid in water and (B) 0.1 % formic acid in acetonitrile. They
184 demonstrated that flavonoids such as astilbin exhibit stronger signal responses in negative ion
185 mode than positive ion mode. Therefore, the negative ion mode was used in this study. First, the
186 chromatographic conditions of this method were optimized. Given the logP values of astilbin and
187 *epi*-DPA-G (1.09 and -1.27 respectively), the retention time of astilbin on the C18 column was
188 expected to be higher than that of *epi*-DPA-G. The values were estimated using Chemaxon
189 software (ChemAxon Kft., Budapest, Hungary) at <https://www.chemaxon.com/marvin/sketch/>.
190 Acetonitrile was used as the organic part of the mobile phase because it is more suitable for faster
191 elution of the low polarity polyphenols. Formic acid was added to the mobile phase in order to

192 decrease the pH and improve the shape of the peaks and the chromatographic resolution, even
193 though it may inhibit the ionization of acidic compounds in the matrix (Chen, Lu, & Zhao, 2014).
194 Three gradients were tested on five different end-capped C18 columns (Hypersil Gold, HSST3,
195 BEH, Synchronis and Kinetex) and separation of astilbin and *epi*-DPA-G was achieved in all
196 cases. The efficiency of the gradients and columns were evaluated by comparing the validation
197 parameters (RSD, LOQ and LOD) for the injection of calibration solutions ranging from 0.02 to
198 10 mg/L. For each column, gradients **I** and **II** gave almost similar results, whereas gradient **III**
199 was the best for separating *epi*-DPA-G and astilbin (**Tables 2 and 3**). This is probably because
200 gradient **III** started with 90 % of 0.1 formic acid in water instead of 95 or 98 %, which
201 minimized the retention of the analytes and also reduced the clustering of peaks, especially when
202 analyzing the wine matrix. In addition, by extending the organic phase from 1 min to 7.5 min, a
203 better separation was achieved due to better interaction of the polar compounds with the
204 stationary phase, as illustrated by the better reproducibility of retention time and sensitivity.
205 The different tested C18 columns are end-capped. However, due to their manufacturing process
206 and geometry, their retention of analytes and their reproducibility and sensitivity are not the
207 same.
208 Hypersil Gold C18, used in our previous qualitative study (Crétin et al., 2019), is known to retain
209 compounds over a wide range of polarity. It has a proprietary derivatization system and has a
210 highly pure silica end cap that the manufacturers claim reduces peak tailing and improves
211 efficiency, particularly at very low pH (2-5). It is therefore used as stationary phase in LC-MS
212 applications (Fanigliulo et al., 2011). On the other hand, the T3 bonding of high silica strength
213 HSS uses a trifunctional C18 alkyl with a 1.8 µm bonded phase at a ligand density that promotes
214 the retention of small, water-soluble polar organic compounds and aqueous mobile phase
215 compatibility, so HSST3 could also be suitable for this study. The BEH C18 column incorporates

216 trifunctional ligand bonding chemistries on the 1.7 μm BEH particles based on new end-capping
217 processes that ensure good peak shape for basic analytes (Gritti & Guiochon, 2013; New &
218 Chan, 2008). Synchronis C18 has been engineered to provide good reproducibility thanks to its
219 highly pure and high surface area silica, dense bonding and double endcapping that minimizes
220 secondary interactions (« Column range delivers reproducibility », 2010). Indeed, good
221 reproducibility was obtained when using this column (**Tables 2** and **3**). Finally, Kinetex C18 is a
222 uniform porous silica layer grown around a spherical solid silica core. This combination of
223 precise particle architecture provides dramatic leaps in performance and increases the rate of
224 mass transfer by decreasing the effects of diffusion and reducing losses in efficiency (Gritti et al.,
225 2017).

226 In this study, Hypersil Gold C18 was the most suitable column to quantify the targeted
227 compounds, especially when using gradient **III**. An efficient and rapid separation with good
228 resolution was obtained since *epi*-DPA-G and astilbin eluted at 1.4 and 3.6 min, respectively,
229 which is important for routine analysis. The ionization parameters were optimized by automatic
230 tune for astilbin and *epi*-DPA-G. For each sample analyzed, extracted ion chromatograms (XIC)
231 were built in a 5 ppm window around the empirical formula of each compound. *Epi*-DPA-G with
232 a composition of $\text{C}_{21}\text{H}_{32}\text{O}_{10}$ presented a HRMS spectrum with a quasi-molecular $[\text{M}-\text{H}]^-$ ion at
233 m/z 443.19028, while astilbin with the empirical formula $\text{C}_{21}\text{H}_{22}\text{O}_{10}$ had a $[\text{M}-\text{H}]^-$ ion at m/z
234 449.10681.

235 The validation studies were performed in accordance with the regulatory guidelines stipulating
236 that a method used for the quantitative measurement of analytes should be reliable and
237 reproducible for the intended use (Pereira et al., 2018)
238 ([http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-](http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf)
239 [4252fnl.pdf](http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf)). Results summarized in *section 3.2.* and in **Tables 2** and **3** demonstrate good

240 reproducibility for all the columns with the best value obtained with Hypersil, for which intra-
241 day RSD_{tr} was 0.20 % for *epi*-DPA-G and astilbin. To perform the quantification, other
242 validation parameters such as linearity, RSD_A , LOQ, LOD and recovery were also evaluated.

243

244 3.2. *Additional validation parameters*

245 3.2.1. *Linearity*

246 The parameters of the standard calibration curves obtained from the average concentration of *epi*-
247 DPA-G and astilbin at seven different levels, using three gradients and on five C18 columns are
248 presented in **Tables 2** and **3**. The resulting correlation coefficient (r^2) makes it possible to
249 estimate the linearity of the curve obtained. Depending on the columns and the gradients, r^2
250 values were obtained from 0.9837 to 0.9999 for *epi*-DPA-G and from 0.8542 and 0.9992 for
251 astilbin in the concentration range 0.02 - 10 mg/L. This range was chosen for the linearity study,
252 since it included the concentrations of *epi*-DPA-G and astilbin estimated in the tested red wines.
253 For *epi*-DPA-G, the calibration curves were satisfactorily linear, especially for Hypersil and
254 HSST3 with all gradients. For the three other columns, the best results were obtained with
255 gradients **I** and **II**. For astilbin, the correlation coefficients (r^2) were strongly affected by the
256 column and the best values were obtained with Hypersil ($r^2 \geq 0.9980$ for all gradients) and, to a
257 lesser extent, with Kinetex ($r^2 \geq 0.9927$).

258 3.2.2. *LOD and LOQ*

259 LOQ and LOD (**Tables 2** and **3**) were evaluated using a linearity approach. For both compounds,
260 the best sensitivity was obtained when using gradient **III** with Hypersil. In these conditions, LOQ
261 was 18 and 20 $\mu\text{g/L}$ for *epi*-DPA-G and astilbin, respectively. Extracted ion chromatograms of

262 *epi*-DPA-G and astilbin at 10 µg/L (similar to that of LOD) are presented in the **supporting**
263 **information (Figure S-1).**

264 **3.2.3. Intra- and inter-day precision (RSD)**

265 RSD_A was evaluated for *epi*-DPA-G and astilbin in the different chromatographic conditions.
266 Good intra-day repeatabilities were obtained for all columns but with a preference for Hypersil,
267 for which RSD_A was 3.0% and 2.0% for *epi*-DPA-G and astilbin, respectively (**Tables 2** and **3**).
268 In these conditions, inter-day repeatabilities on retention times and peak areas for *epi*-DPA-G
269 and astilbin evaluated were lower than 3.5 % (n=3).

270 **3.2.4. Recovery**

271 Based on the previous linearity, sensitivity and repeatability results, gradient **III** and Hypersil
272 columns were selected for the quantitative study. Recovery was evaluated for both compounds in
273 these conditions. Three known concentrations of *epi*-DPA-G and astilbin (100 µg/L; 500 µg/L
274 and 1 mg/L) were spiked in PO1999b, SES2001 and VPCR1992. The recovery values ranged
275 from 89 to 99 %, which meets the requirements of the guidelines (**Table 4**). Therefore, this
276 method is suitable for quantifying *epi*-DPA-G and astilbin in red wine.

277 278 **3.3. Application of method for quantification of *epi*-DPA-G and astilbin in various French** 279 **commercial red wines**

280 After validating the method by using gradient **III** and the Hypersil column, several vintages of French red
281 wines from four wine regions and 15 appellations were assayed (**Table 1**). The concentrations of *epi*-
282 DPA-G and astilbin quantified in wine were determined from the calibration curve of the purified
283 standards and by considering the dilution factor. *Epi*-DPA-G was detected at 1.40 min and astilbin at 3.62

284 min. Therefore, this demonstrates the selectivity of the method to identify and quantify *epi*-DPA-G and
285 astilbin in wine. However, additional peaks with the same mass and molecular formula were
286 present at 2.53, 3.45 and 3.96 min, suggesting the possible presence of astilbin isomers. These
287 additional peaks were almost present in the different vintages of the red wine tested and could be
288 separated by using gradient **III**. An example of an extracted ion chromatogram (XIC) of *epi*-
289 DPA-G and astilbin present in a PO1999b and obtained by using gradient **III** on Hypersil C18 is
290 illustrated in **Figure 1**.

291 As shown in the **supporting information (Table S-1)**, *epi*-DPA-G and astilbin were observed in all
292 wines, at concentrations varying strongly according to the origins and the vintages. *Epi*-DPA-G
293 concentrations ranged from 1.2 to 14.7 mg/L with a mean value of 7.3 mg/L. The lowest quantity
294 of *epi*-DPA-G was present in CL2013 and the highest quantity in CL1923. Astilbin
295 concentrations ranged from 0.5 mg/L (in MA1990) to 42.6 mg/L (in CL2015) with a mean value
296 of 8.1 mg/L. Box plots of CL showed a range of *epi*-DPA-G from 1.2 to 14.7 mg/L and a range
297 of astilbin from 8.5 to 42.8 mg/L (**Figure 2**). Therefore, *epi*-DPA-G and astilbin are highly
298 present in CL.

299 In this study, *epi*-DPA-G was quantified for the first time in wine. Moreover, astilbin
300 concentrations obtained were in the same range of those obtained in the literature (K. Trousdale
301 & L. Singleton, 1983; Landrault et al., 2002). On the other hand, the effect of vintage on astilbin
302 concentrations had never been described until now. The analysis of 16 vintages of the same
303 estate (Clos des Lambrays) revealed high concentrations of both compounds in one-century-old
304 wines, which suggests that they are not significantly degraded over time.

305

306 **Conclusion**

307 An LC-FTMS method has been developed to identify and quantify two sweet molecules present
308 in wines: *epi*-DPA-G and astilbin. Five columns and three gradients were tested to optimize the
309 conditions of analysis. The method is satisfactory in terms of sensitivity, linearity, repeatability
310 and recovery and was applied successfully to quantify *epi*-DPA-G and astilbin in several
311 commercial red wines. *Epi*-DPA-G was quantified in wine for the first time. Both compounds
312 were present at concentrations ranging from a few mg/L to a few tens of mg/L. The presence of
313 high amounts in one-century-old wines suggests the relative stability of both compounds over
314 time. Therefore, this method can now be used to study the effect of grape varieties, origins and
315 maturity on the presence of these sweet compounds. The development of this method brings a
316 new tool that will be useful to investigate the influence of viticultural and enological parameters
317 on the taste of wine. Finally, some astilbin isomers never identified until now in wine were
318 detected in some samples. Future work will focus on the isolation, structural elucidation and
319 sensory assessment of these compounds to determine their potential contribution to sweetness in
320 dry wines.

321

322 **Acknowledgements**

323 *The authors declare that there are no conflicts of interest.*

324

325

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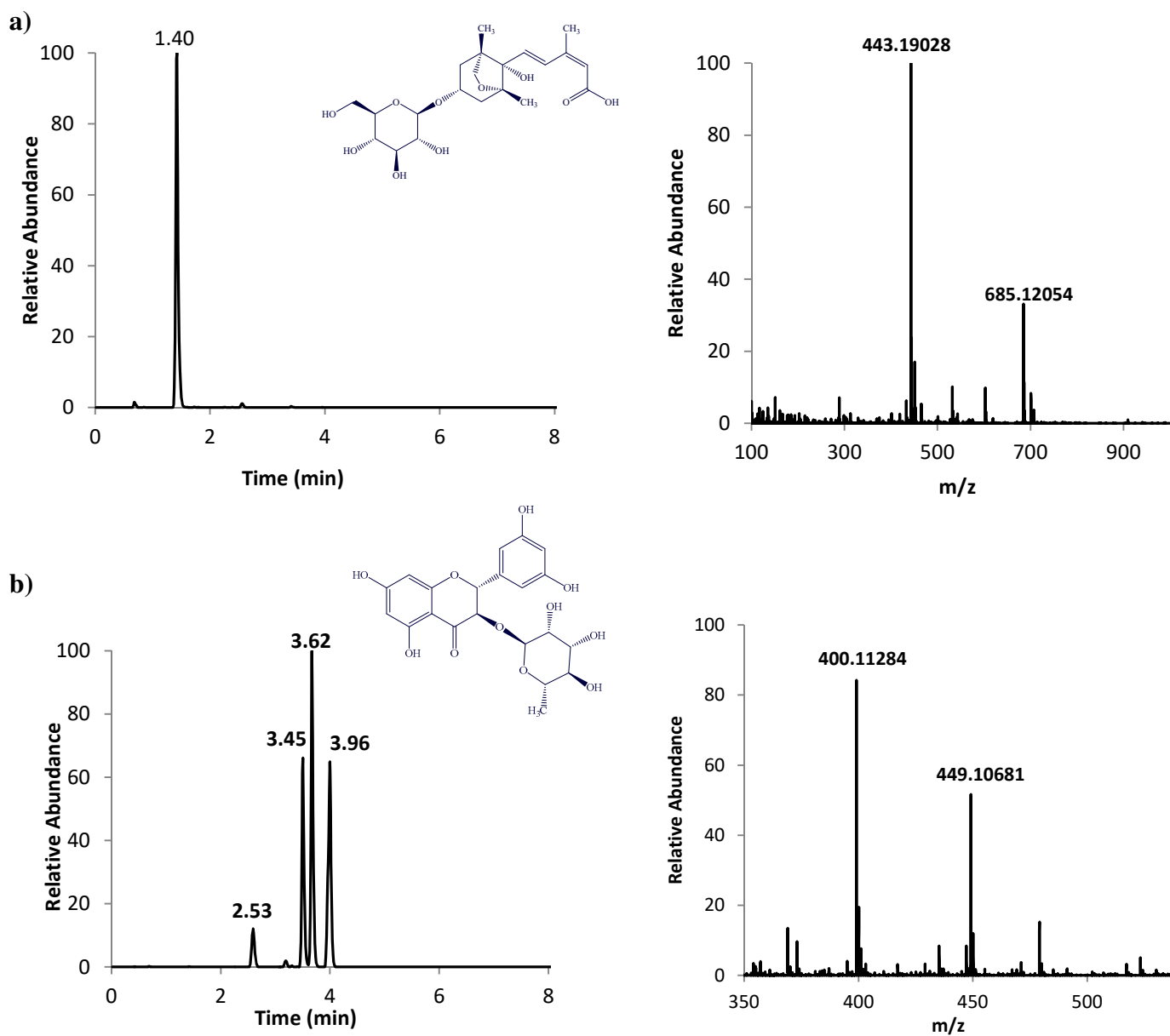
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422

Figure 1: Extracted ion chromatogram and mass spectra of a) *epi*-DPA-G and b) astilbin present in a PO1999b.



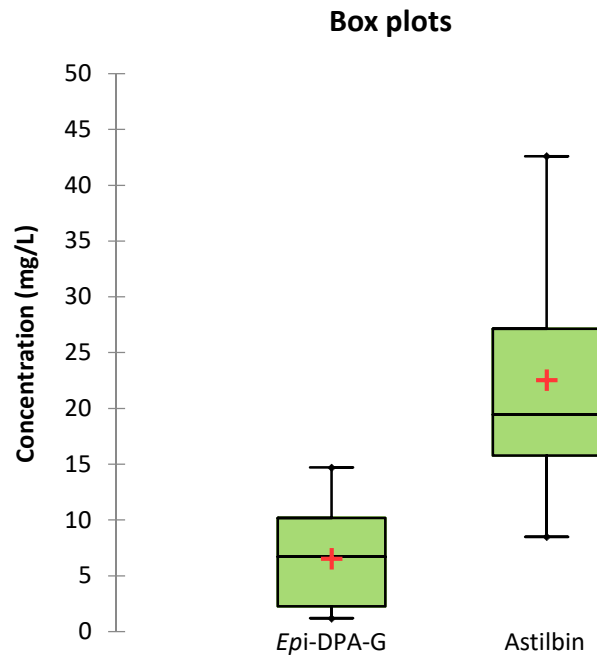


Figure 2: Box plots of *epi*-DPA-G and astilbin in several vintages of CL.

Table 1: Origin, vintage and grape varieties of the commercial wines used for quantification assays

Apellation	Vintage	Grape variety*	Region	
Pomerol	1981	M, CF, Ma	Bordeaux	PO1981a
Pomerol	1981	M, CF	Bordeaux	PO1981b
Pomerol	1981	M, CF	Bordeaux	PO1981c
Pomerol	1988	M, CF	Bordeaux	PO1988
Pomerol	1998	M, CF, CS	Bordeaux	PO1998
Pomerol	1999	M, CF	Bordeaux	PO1999a
Pomerol	1999	M, CF	Bordeaux	PO1999b
Pomerol	2007	M, CF	Bordeaux	PO2007a
Pomerol	2007	M	Bordeaux	PO2007b
Pomerol	2007	M, CF	Bordeaux	PO2007c
Pomerol	2008	M, CF	Bordeaux	PO2008
Saint-Julien	1998	CS, M, CF	Bordeaux	SJ1998a
Saint Julien	1998	CS, M, CF, PV	Bordeaux	SJ1998b
Saint Julien	1998	CS, M, CF, PV	Bordeaux	SJ1998c
Saint Julien	1999	CS, M, CF	Bordeaux	SJ1999
Saint Julien	2000	CS, M, CF	Bordeaux	SJ2000
Saint Julien	2002	CS, M, CF, PV	Bordeaux	SJ2002a
Saint Julien	2002	M, CS, CF	Bordeaux	SJ2002b
Saint Julien	2004	CS, M, CF	Bordeaux	SJ2004
Saint Julien	2007	CS, M	Bordeaux	SJ2007a
Saint Julien	2007	CS, M, CF, PV	Bordeaux	SJ2007b
Saint Julien	2008	CS, M, CF, PV	Bordeaux	SJ2008a
Saint Julien	2008	CS, M, CF	Bordeaux	SJ2008b
Saint Julien	2008	CS, M, CF, PV	Bordeaux	SJ2008c
Saint Emilion Grand cru	2003	M, CF	Bordeaux	SE2003
Saint Emilion Grand cru	2006	M, CF	Bordeaux	SE2006
Saint Emilion Grand Cru	2007	CF, M	Bordeaux	SE2007

Saint Emilion Grand cru	2013	M, CF	Bordeaux	SE2013
Saint-Emilion Grand Cru	2014	M, CF, CS	Bordeaux	SE2014
Margaux	1990	CS, M	Bordeaux	MA1990
Margaux	1997	CS, M	Bordeaux	MA1007
Margaux	2002	CS, M, CF, PV	Bordeaux	MA2002a
Margaux	2002	CS, M	Bordeaux	MA2002b
Pauillac	1999	CS, M, CF	Bordeaux	PA1999
Pauillac	2002	CS, M, CF	Bordeaux	PA2002
Pauillac	2005	CS, M, PV	Bordeaux	PA2005
Medoc	2004	M, CS, CF	Bordeaux	ME2004
Medoc	2009	M, CS, CF	Bordeaux	ME2009
Medoc	2014	M, CS, CF	Bordeaux	ME2014
Haut-Medoc	1983	M, CS, PV, CF	Bordeaux	HM1983
Haut-Medoc	1984	M, CS, PV, CF	Bordeaux	HM1984
Pessac-Léognan	1994	CS, M	Bordeaux	PL1994
Pessac-Léognan	2006	CS, M, PV	Bordeaux	PL2006
Pessac-Léognan	2008	CS, M, PV	Bordeaux	PL2008
Graves	2006	CS, M	Bordeaux	GR2006
Graves	2008	CS, M	Bordeaux	GR2008
Premières Côtes de Bordeaux	2007	M, PV, CS	Bordeaux	PCB2007
Premières Côtes de Bordeaux	2008	M, PV, CS	Bordeaux	PCB2008
Saint Estèphe	2001	M, CS, PV, CF	Bordeaux	SES2001
Clos des Lambrays	1918	PN	Bourgogne	CL1918
Clos des Lambrays	1919	PN	Bourgogne	CL1919
Clos des Lambrays	1923	PN	Bourgogne	CL1923
Clos des Lambrays	1934	PN	Bourgogne	CL1934
Clos des Lambrays	1937	PN	Bourgogne	CL1937
Clos des Lambrays	1946	PN	Bourgogne	CL1946
Clos des Lambrays	1949	PN	Bourgogne	CL1949
Clos des Lambrays	1950	PN	Bourgogne	CL1950
Clos des Lambrays	1967	PN	Bourgogne	CL1967

Clos des Lambrays	1972	PN	Bourgogne	CL1972
Clos des Lambrays	1997	PN	Bourgogne	CL1997
Clos des Lambrays	2003	PN	Bourgogne	CL2003
Clos des Lambrays	2005	PN	Bourgogne	CL2005
Clos des Lambrays	2013	PN	Bourgogne	CL2013
Clos des Lambrays	2015	PN	Bourgogne	CL2015
Clos des Lambrays	2017	PN	Bourgogne	CL2017
Vin de Pays des Collines Rhodaniennes	1992	S	Rhône Valley	VPCR1992
Crozes Hermitage	2014	S	Rhône Valley	CH2014
Vin de Pays d'Oc	2001	M	Languedoc Roussillon	VPO2001

* Cabernet Franc : CF ; Cabernet Sauvignon : CS ; Malbec : Ma ; M : Merlot ; Petit Verdot : PV ; Pinot Noir : PN ; Syrah : S

Table 2: Evaluation of validation parameters of *epi*-DPA-G on five columns using three different gradients

Column	Gradient	LOQ ($\mu\text{g/L}$)	LOD ($\mu\text{g/L}$)	RSD _{tr} (%)	RSD _A (%)	Linearity	
Hypersil	I	1930	643	0.6	3.5	$r^2=0.9992$	$a=4 \times 10^6$ $b=-1.9 \times 10^5$
	II	150	50	0.5	3.0	$r^2=0.9992$	$a=8 \times 10^6$ $b=-1.9 \times 10^5$
	III	18	6	0.2	3.0	$r^2=0.9998$	$a=4 \times 10^6$ $b=-9.9 \times 10^4$
HSST3	I	1039	346	1.1	3.4	$r^2=0.9997$	$a=3 \times 10^6$ $b=4.2 \times 10^4$
	II	54	18	0.5	12	$r^2=0.9973$	$a=2 \times 10^6$ $b=2.7 \times 10^4$
	III	102	33	0.5	3.0	$r^2=0.9960$	$a=4 \times 10^6$ $b=2.1 \times 10^6$
BEH	I	280	93	1.4	4.6	$r^2=0.9995$	$a=3 \times 10^6$ $b=-3.3 \times 10^5$
	II	39	13	1.3	3.0	$r^2=0.9941$	$a=2 \times 10^6$ $b=-2.9 \times 10^5$
	III	65	21	0.5	7.0	$r^2=0.9837$	$a=3 \times 10^6$ $b=-1.9 \times 10^5$
Synchronis	I	1940	647	1.4	5.7	$r^2=0.9999$	$a=3 \times 10^6$ $b=-4.3 \times 10^5$
	II	247	83	0.5	3.4	$r^2=0.9950$	$a=1 \times 10^6$ $b=-2.9 \times 10^5$
	III	585	195	0.4	6.0	$r^2=0.9898$	$a=2 \times 10^6$ $b=3.8 \times 10^5$
Kinetex	I	2500	833	1.9	5.0	$r^2=0.9992$	$a=2 \times 10^6$ $b=-9.7 \times 10^4$
	II	240	80	0.4	8.0	$r^2=0.9981$	$a=2 \times 10^6$ $b=-2.2 \times 10^5$
	III	95	31	1.2	4.3	$r^2=0.9844$	$a=3 \times 10^6$ $b=-1 \times 10^6$

Table 3: Evaluation of validation parameters of astilbin on five different columns using three different gradients

Column	Gradient	LOQ (µg/L)	LOD (µg/L)	RSD _{tr} (%)	RSD _A (%)	Linearity	
Hypersil	I	45	15	0.2	7.0	r ² =0.9992	a=2.9x10 ⁵ b=-1.1x10 ⁵
	II	29	10	0.1	5.0	r ² =0.9992	a=6.1x10 ⁵ b=-8.2x10 ⁴
	III	20	7	0.1	2.0	r ² =0.9980	a=6.8x10 ⁵ b=-1.1x10 ⁵
HSST3	I	29	10	0.3	8.0	r ² =0.9945	a=2.8x10 ⁵ b=-2.3x10 ³
	II	27	9	0.2	12.0	r ² =0.9944	a=3.1x10 ⁵ b=9.4x10 ³
	III	23	8	0.6	6.0	r ² =0.9933	a=7.6x10 ⁵ b=1.1x10 ⁵
BEH	I	124	40	0.2	10.9	r ² =0.8542	a=1.1x10 ⁵ b=-1.7x10 ⁵
	II	100	34	0.3	8.1	r ² =0.8886	a=9.1x10 ⁴ b=-4.9x10 ⁴
	III	320	114	0.9	5.2	r ² =0.9205	a=5.1x10 ⁵ b=-4.2x10 ⁵
Synchronis	I	124	38	0.2	10.1	r ² =0.9542	a=1.2x10 ⁵ b=-1.4x10 ⁵
	II	340	120	0.4	19.0	r ² =0.9385	a=5.5x10 ⁴ b=-3.7x10 ⁴
	III	198	66	0.8	4.0	r ² =0.9898	a=1.6x10 ⁵ b=6.9x10 ⁴
Kinetex	I	120	40	0.2	15.0	r ² =0.9927	a=1.8x10 ⁵ b=-2.5x10 ³
	II	240	80	0.2	4.0	r ² =0.9982	a=2.2x10 ⁵ b=-4.5x10 ⁴
	III	203	67	3.0	9.0	r ² =0.9973	a=4.4x10 ⁵ b=-9.6x10 ⁴

Table 4: Recovery (%) of *epi*-DPA-G and astilbin in PO1999b, SES2001 and VPCR1992

Recovery (%) Spiked concentrations (µg/L)	PO1999b		SES2001		VPCR1992	
	<i>Epi</i> -DPA-G	Astilbin	<i>Epi</i> -DPA-G	Astilbin	<i>Epi</i> -DPA-G	Astilbin
100	94	89	91	89	95	93
500	89	96	92	95	92	89
1000	95	90	97	91	99	90