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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*- $\beta$ -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*- $\beta$ -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  $\mu\text{m}$ -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  $\mu\text{m}$ ), Synchronis™ (100 mm x 2.1 mm, 1.7  $\mu\text{m}$ ) from Thermo  
118 Fisher Scientific, High Silica Strength (HSST3; 100 mm x 2.1 mm, 1.8  $\mu\text{m}$ ), Bridged  
119 Ethylsiloxane/silica Hybrid (BEH; 100 mm x 2.1 mm, 1.7  $\mu\text{m}$ ) from Waters and Kinetex (100  
120 mm x 2.1 mm, 1.7  $\mu\text{m}$ ) from Phenomenex. All the columns were protected by a guard column.  
121 Five  $\mu\text{L}$  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 10<sup>6</sup>. 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](http://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  $\mu$ g/L, 500  $\mu$ 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  $\mu\text{m}$  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  $\mu\text{m}$  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<sub>A</sub> 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<sub>A</sub> 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

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326 **References**

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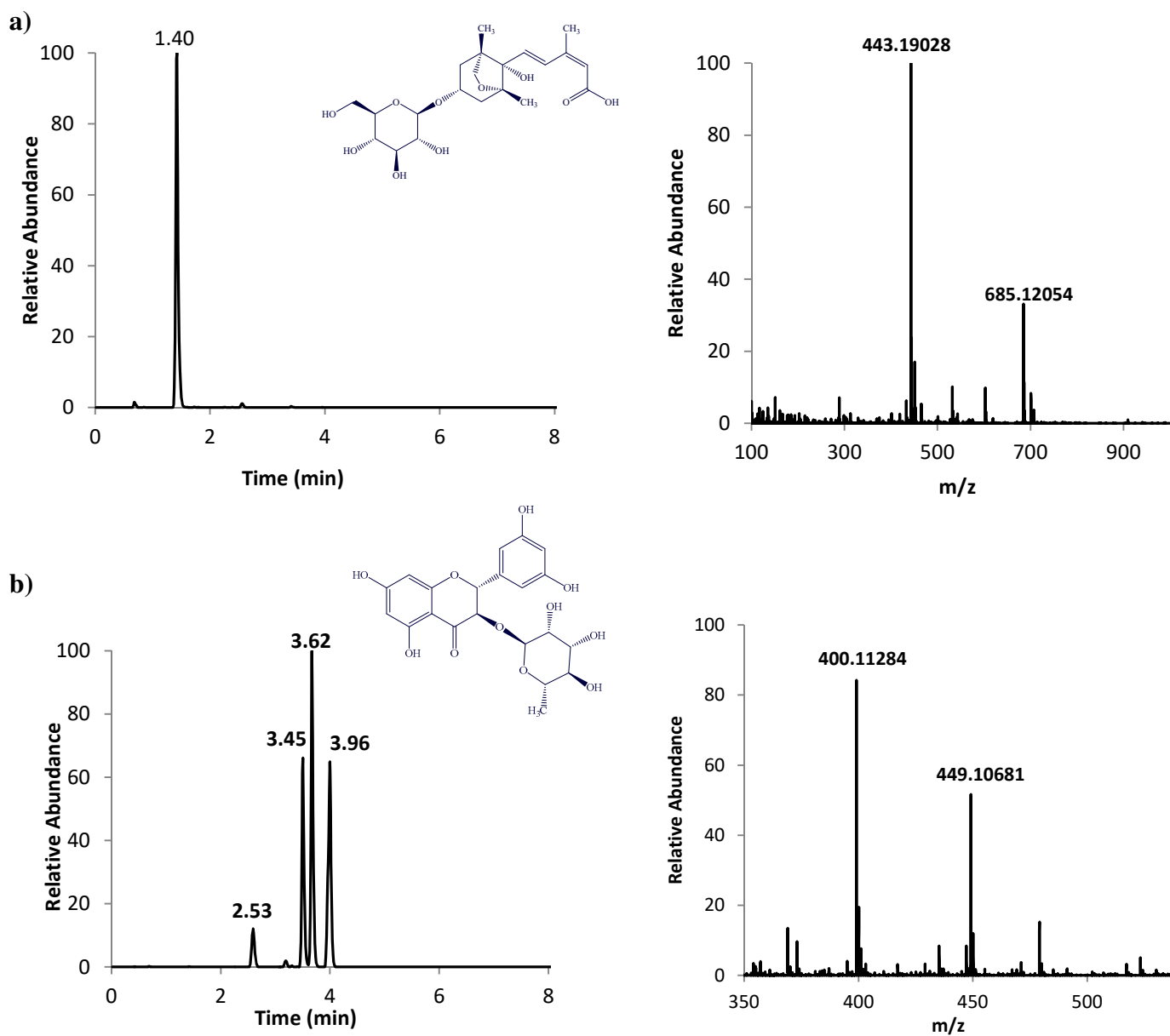
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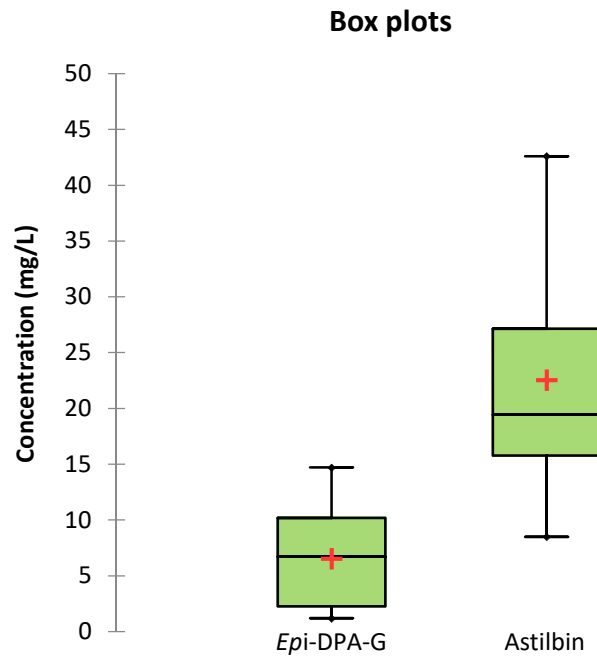
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422

**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

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



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

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

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

**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