Profiling of *Vitis vinifera* L. canes (poly)phenolic compounds using comprehensive two-dimensional liquid chromatography.

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21 **ABSTRACT.**

22 Grapevine canes, a pruning-derived by-product, possess a great amount of bioactive
23 (poly)phenolic compounds belonging to different chemical classes, thus, having a good
24 potential for further valorization. However, in order to properly design valorization
25 strategies, the precise chemical composition of this material has to be known. Up to
26 now, this chemical characterization has been based on analysis of different groups of
27 components individually, due to difficulties related to their huge chemical variability. In
28 this work, a comprehensive two-dimensional liquid chromatography-based method (LC
29 × LC) is developed to obtain the profiles of (poly)phenolic compounds present in
30 grapevine canes from several varieties. Three different set-ups have been tested and
31 compared; the combination of diol and C_{18} columns produced the best results, allowing
32 the characterization of the (poly)phenolic profile in around 80 min. This way, 81
33 different components were detected in the samples; most of them could be tentatively
34 assigned using the information provided by the DAD and MS detectors employed.
35 Indeed, it has been possible to detect in a single run components belonging to
36 stilbenoids, procyanidins and prodelphinidins of varying degrees of polymerization,
37 some of them not formerly described in this natural source. The method has shown
38 extremely good separation capabilities, and is characterized by high effective peak
39 capacity (842) and orthogonality (A_0 = 78%). The obtained results demonstrate that *Vitis*
40 *vinifera* L. canes may retain a great potential to be used as an underexploited natural
41 source of bioactive compounds, with potential applications in different fields.

42

43 **Keywords:** Grapevine canes; LC × LC; Phenolic compounds; Proanthocyanidins;
44 Stilbenoids

45

46 **1. INTRODUCTION.**

47 Management of agricultural and food-related by-products and wastes is an important
48 issue nowadays worldwide. Industrial practices related to food production are
49 responsible for the generation of a huge amount of unwanted materials at different
50 levels. Traditionally, these wastes have been reused for energy generation and/or feed
51 production [1]. Nevertheless, this approach is clearly not efficient enough to deal with
52 such a high amount of by-products. For this reason, different alternatives have appeared
53 in the last years proposing new ways for the valorization of agricultural and food
54 industry by-products [2], considering that a significant part of those wastes are still rich
55 on interesting components, such as bioactives. Indeed, at present, the complete
56 valorization of all the residues and by-products generated in a particular production
57 chain is ideally sought through the application of the modern concept of biorefinery [3].
58 Among the different agrofood-related by-products, grapevine (*Vitis vinifera* L.) canes
59 are a promising source of different bioactive components, basically, phenolic
60 compounds. Canes are a pruning residue which is not processed for extensive
61 valorization as they are normally burnt or composted [4]. Among the bioactives present
62 in this material, stilbenoids are commonly pointed out [5], although others such as
63 proanthocyanidins are also present. Stilbenoids are non-flavonoid phenolic compounds
64 which are related to defense mechanisms in plants as a response to different stresses.
65 The basic structure of those found in grapevines are based on (*E*)-resveratrol (3,5,4
66 trihydroxystilbene) chemical structure, which is also the most abundant compound in
67 grapevine canes after post-pruning storage. However, reactions such as
68 photoisomerization, glycosylation and oligomerization are responsible for the complex
69 chemical pattern that can be natively found in the plant [6], including monomers ((*E*)-
70 piceatannol, (*E*)-piceid), dimers ((*E*)-ε-viniferin, (*E*)-ω-viniferin, ampelopsin A,

71 vitisinol C), trimers ((*E*)-miyabenol C), and tetramers ((*E*)-vitisin B, (*Z*)-vitisin B,
72 hopeaphenol, isohopeaphenol), among others. Moreover, the levels of (*E*)-resveratrol
73 and some other related minor stilbenoids are strongly dependent on storage conditions
74 of canes (time, temperature) after pruning. It has been observed that pruning triggers a
75 very significant increase in stilbenoid levels, mainly (*E*)-resveratrol, in grapevine canes
76 [6,7], which is induced by the stress affecting the vegetal material during post-pruning
77 storage. The increase of the activity of the stilbenoid synthesizing enzyme during this
78 period has been already reported [7], indicating that the biosynthesis is activated.
79 Interestingly, this increase is not observed if the vegetal material is not cut or if it is kept
80 frozen or ground soon after collection [5,6]. Different beneficial health effects and
81 bioactive activities have been ascribed to *(E)*-resveratrol as well as to other stilbenoids
82 [8], thus, highlighting the interest on these natural components.
83 On the other hand, proanthocyanidins are flavan-3-ol polymers which can be linked
84 through multiple ways and degrees of polymerization, giving rise to extremely complex
85 patterns [9]. As for stilbenoids, proanthocyanidins are regarded as responsible for a
86 number of bioactivities, including antioxidant, hepatoprotective, anti-inflammatory,
87 antibacterial or anticancer effects, among others [10]. Different proanthocyanidins,
88 mainly procyanidins, have been already described in grapevine [11], although the
89 natural chemical variability may still be concealed due to difficulties in their analysis.
90 Consequently, the presence of this complex array of (poly)phenolic compounds makes
91 grapevine canes a potentially interesting material for the development of valorization
92 processes.
93 However, to produce an efficient valorization of wastes, not only environmentally
94 friendly extraction and processing techniques are needed to obtain the compounds of
95 interest, but also an exhaustive chemical characterization of those materials is required.

In fact, it is of utmost importance to precisely know the chemical composition of a particular by-product in order to devise strategies for its valorization. In this regard, the already mentioned extremely complex pattern on bioactives present on grapevine canes implies that the typically used one-dimensional separation approaches may not provide the separation and identification power enough to reveal more in detail the chemical composition of these wastes. It is precisely on this kind of complex natural samples where comprehensive two-dimensional liquid chromatography (LC × LC) may provide with the required additional separation capabilities. LC × LC is based on the coupling of two independent separation mechanisms that allow significant improvements on resolving power and peak capacity [12]. By using this on-line approach, the entire sample is subjected to two independent separation mechanisms continuously; although different combinations between separation mechanisms may be applied, the one involving hydrophilic interaction chromatography (HILIC) coupled to reversed phase (RP) separations has shown a very good potential for polyphenols analysis [13]. In any case, the application of this coupling is not straightforward due to multiple factors that should be optimized [14-16], being one of the most important the transfer from the first dimension (^{1}D) eluent to the second dimension (^{2}D) continuously, due to solvent incompatibility. Although, this technique has been already employed for the analysis of different types of polyphenols and matrices [17], up to now, it has not been used for the profiling of grapevine canes. Thus, the aim of this work is to profile and characterize the complex mixture of (poly)phenolic compounds contained in grapevine canes, mainly stilbenoids and proanthocyanidins, in a single run through the use of a HILIC × RP method coupled to tandem mass spectrometry. The developed method is then applied to reveal differences on the chemical composition between two red grapevine varieties stored for 3 months after pruning to foster an accumulation of stilbenoids.

121

122 **2. MATERIALS AND METHODS.**

123 **2.1. Samples and chemicals.**

124 Grapevine (*Vitis vinifera L.*) canes from the variety *Pinot Noir* were collected from Itata
125 Valley (Concepción, Chile) and canes from the variety *Cabernet Sauvignon* were from
126 Maipo Valley (Santiago, Chile) in the winter of 2013. After pruning, both samples were
127 stored at room temperature during three months. Then, the grapevine canes were ground
128 and frozen at -20°C. Extraction of (poly)phenolic compounds from dried canes was
129 carried out by solid/liquid extraction. Briefly, 50 mL of acetone/water (80:20, *v/v*) were
130 added to 5 g of ground grapevine canes. The solution was sonicated (Elma, Singen,
131 Germany) for 15 min. After that, the mixture was kept in the darkness during 2 h and
132 then it was again sonicated for 15 min. Finally, the solution was centrifuged for 20 min
133 at 8000 rpm, the acetone was evaporated under vacuum (Rotavapor R-210, Büchi
134 Labortechnik AG, Flawil, Switzerland) and lastly, the aqueous extract was freeze-dried
135 (Labconco Corporation, MO).

136 HPLC grade methanol, acetonitrile and acetone were purchased from VWR Chemicals
137 (Barcelona, Spain), whereas acetic and formic acids were acquired from Sigma-Aldrich
138 (Madrid, Spain) and ammonium acetate was from Panreac (Barcelona, Spain). Water
139 employed was Milli-Q grade obtained from a Millipore system (Billerica, MA).

140

141 **2.2. Instrumentation.**

142 The LC × LC-DAD instrumentation consisted on a first dimension (^{1}D) composed by an
143 Agilent 1200 series liquid chromatograph (Agilent Technologies, Santa Clara, CA)
144 equipped with an autosampler. In order to obtain more reproducible low flow rates and
145 to minimize the gradient delay volume of the pump, a Protecol flow-splitter (SGE

146 Analytical Science, Milton Keynes, UK) was placed between the ^{1}D pump and the
147 autosampler. Additionally, a LC pump (Agilent 1290 Infinity) performed the second
148 dimension (^{2}D) separation. Both dimensions were connected by an electronically-
149 controlled two-position ten-port switching valve (Rheodyne, Rohnert Park, CA, USA)
150 acting as modulator equipped with two identical 30 µL injection loops. Modulation time
151 of the switching valve was 1.3 min. A diode array detector was coupled after the second
152 dimension in order to register every ^{2}D analysis. Besides, an Agilent 6320 Ion Trap
153 mass spectrometer equipped with an electrospray interface working under negative
154 ionization mode was coupled in series using the following conditions: dry temperature,
155 350 ºC; dry gas flow rate, 12 L min^{-1}; nebulization pressure, 40 psi; mass range, *m/z* 90-
156 2200 Da; ultra scan mode (26000 *m/z* /s). The LC data were elaborated and visualized
157 using LC Image software (version 1.0, Zoex Corp., Houston, TX).
158
159 **2.3. LC × LC separation conditions.**
160 The ^{1}D separation was optimized using three sets of columns. The best conditions for
161 each column after optimization were:
162 i) ZIC-HILIC column (150 × 1 mm, 3.5 µm, Merck, Darmstadt, Germany) eluted using
163 acetonitrile (A) and 10 mM ammonium acetate pH 5 (B) as mobile phases, using the
164 following gradient at 15 µL min^{-1}: 0 min, 3% B; 5 min, 3% B; 10 min, 5% B; 15 min,
165 10% B; 30 min, 20% B; 45 min, 20% B; 50 min, 30% B; 60 min, 30% B; 70 min, 40%
166 B; 80 min, 40% B.
167 ii) PEG column (150 × 2.1 mm, 5 µm, Supelco, Bellefonte, CA) eluted using methanol
168 (0.1 % formic acid, A) and water (0.1 % formic acid, B) at 20 µL min^{-1} according to the
169 following gradient: 0 min, 40% B; 50 min, 10% B; 70 min, 2% B.

170 iii) Lichrospher diol-5 (150 × 1.0 mm, 5 µm, HiChrom, Reading, UK) column eluted
171 using acetonitrile (1% formic acid, A) and methanol/10 mM ammonium acetate/acetic
172 acid (95:4:1, B) at 18 µL min^{-1} using the following gradient: 0 min, 2% B; 10 min, 2%
173 B; 15 min, 5% B; 30 min, 20% B; 45 min, 20% B; 50 min, 30% B; 60 min, 30% B; 70
174 min, 40% B; 80 min, 40% B.
175
176 On the ^{2}D, a pentafluorophenyl column (Kinetex PFP column, 50 × 4.6 mm, 2.7 µm,
177 Phenomenex, Torrance, CA, USA) and a C_{18} column (Ascentis Express C_{18} column, 50
178 × 4.6 mm, 2.7 µm, Supelco, Bellefonte, CA) were used. For LC × LC analysis, the C_{18}
179 column was employed under optimized conditions depending on the stationary phase
180 used in ^{1}D, as follows:
181 i) diol×C_{18} and PEG×C_{18} set-ups: water (0.1% formic acid, A) and acetonitrile (0.5%
182 formic acid, B) were selected as mobile phases, eluted at 3 mL min^{-1} using the
183 following gradient: 0 min, 2% B; 0.1 min, 2% B; 0.3 min, 10% B; 0.5 min, 25% B; 0.7
184 min, 40% B; 1 min, 60% B, 1.01 min, 2% B.
185 ii) ZIC-HILIC×C_{18} set-up: mobile phases employed were composed by water (0.1%
186 formic acid, A) and acetonitrile (0.5% formic acid, B) and were eluted at 3 mL min^{-1}
187 using the following gradient: 0 min, 0% B; 0.1 min, 2% B; 0.3 min, 5% B; 0.5 min,
188 15% B; 0.7 min, 25% B; 1 min, 50% B; 1.01, 0% B.
189 Independently of the column combinations, 2D analyses were performed maintaining a
190 column temperature of 25 ºC. UV-Vis spectra were collected in the range of 190-550
191 nm using a sampling rate of 20 Hz, while 254, 280 and 330 nm signals were also
192 independently recorded. The effluent from the ^{2}D column was splitted before entering
193 the MS instrument, so that the flow rate introduced in the MS detector was *ca.* 0.6 mL
194 min^{-1}. MS detection was performed as above indicated (section 2.2).

2.4. Calculations.

2.4.1 Peak capacity.

Individual peak capacity for each dimension was calculated according to eq. 1:

$$n_c = 1 + \frac{t_G}{\overline{w}} \qquad (1)$$

where t_G is the gradient time and $\overline{w}$ is the average peak width, equivalent to 4σ. For ^{1}D peak capacity calculations, the average peak width was obtained from 10-15 representative peaks selected along the analysis. Likewise, for ^{2}D peak capacity, as much as possible peaks were considered (14-22 peaks, depending on the analysis). Additionally, ^1n_c was also calculated considering the broadening factor <β>, giving rise to a corrected ^{1}D peak capacity (eq. 2), considering the influence of the deleterious effect of undersampling. To estimate <β>, the sampling time (t_s) as well as the average width of ^{1}D peaks before modulation were considered:

$$^1n_{c,corrected} = \frac{n_c}{\sqrt{1+0.21\left(\frac{t_s}{^1\sigma}\right)^2}} \qquad (2)$$

For each two-dimensional set-up, different peak capacity values were estimated. First of all, theoretical peak capacity was obtained following the so-called product rule, using eq. 3, considering the individual peak capacities obtained in each dimension:

$$^{2D}n_{c,theoretical} = {}^1n_c \times {}^2n_c \qquad (3)$$

As eq. 3 does not take into consideration the deleterious effects due to the modulation process as well as possible undersampling, a more realistic peak capacity value was obtained from the equation proposed by Li et al. [18], denominated here as practical peak capacity (eq. 4):

$$^{2D}n_{c,practical} = \frac{^1n_c \times {}^2n_c}{\sqrt{1+3.35\times\left(\frac{^2t_c\ {}^1n_c}{^1t_G}\right)^2}} \qquad (4)$$

218 being $^{2}t_c$, the ^{2}D separation cycle time, which is equal to the modulation time. This latter
219 equation also includes the <β> parameter accounting for undersampling. Moreover, to
220 more precisely compare among set-ups and in order to evaluate possible peak clusters
221 along the 2D analysis and, thus, to estimate 2D space coverage, the orthogonality
222 degree (A_0) was considered to offer the denominated 2D corrected (also known as
223 effective) peak capacity, as follows:

224 $$^{2D}n_{c,corrected} = {}^{2D}n_{c,practical} \times A_0 \qquad (5)$$

225

226 2.4.2 Orthogonality.

227 Different approaches have been developed and published to quantify the orthogonality
228 degree of a two-dimensional set-up [19]. In the present work, system orthogonality (A_0)
229 was calculated according to the method proposed by Camenzuli and Schoenmakers
230 [20], taking into account the spread of each peak along the four imaginary lines that
231 cross the 2D space forming an asterisk, that is Z_1, Z_2 (vertical and horizontal lines) and
232 Z_-, Z_+ (diagonal lines of the asterisk). Z parameters describe the use of the separation
233 space with respect to the corresponding Z line, allowing to semi-quantitatively diagnose
234 areas of the separation space where sample components are clustered, thus, reducing in
235 practice orthogonality. For the determination of each Z parameter, the S_{Zx} value was
236 calculated, as the measure of spreading around the Z_x line, using the retention times of
237 all the separated peaks in each 2D analysis.

238

239 **3. RESULTS AND DISCUSSION.**

240 Although some previous works dealt with the identification of some stilbenoids [5,21]
241 and proanthocyanidins [22] by one-dimensional reversed phase HPLC in grapevine
242 canes, no comprehensive method has been developed up to now to obtain the

(poly)phenolic profile of this material. Consequently, a LC × LC method has been developed to this aim. Based on the literature and our own experience, as well as considering the nature of the compounds expected to be part of that profile (see Figure 1 for examples), the combination between HILIC × RP could be a promising alternative [17,23-25], although the application of RP × RP has also been explored [17]. To perform a proper method optimization, different conditions have been tested independently, firstly looking at the performance achievable by three different stationary phases in the ^{1}D and then, studying their potential when combined with a C_{18} column in the ^{2}D. This method optimization has been performed considering the available materials and instruments, which impose some important constraints, mainly related to the maximum pressure borne by the equipment (400 bar) as well as to the scanning speed of the available detectors (DAD and MS). Thus, method development has been guided taking some compromises, as described below, not only in terms of theory but also in terms of practice (instrumental limitations). Finally, in order to select the most appropriate set-up for the separation of the grapevine cane samples, the obtained results were critically compared in terms of separation capabilities (overall resolution, peak capacity and orthogonality).

3.1 Separation method optimization.

Unlike other previously investigated samples where a phenolic group of compounds was clearly predominant [23-25], the studied samples in the present work are composed of complex mixtures of varying degrees of polymerization of two different groups of polyphenols, i.e., stilbenoids and proanthocyanidins. Due to this different pattern, several stationary phases compatible with HILIC separations were evaluated for their use in ^{1}D separation, namely, diol, ZIC-HILIC and PEG (polyethylene glycol) columns.

268 Diol stationary phases have repeatedly shown to provide good retention under HILIC
269 mode [17], whereas ZIC-HILIC particles carry zwitterionic functional groups
270 (sulfobetaine) with a charge balance 1:1, also suitable for that separation mode. On the
271 other hand, PEG columns were initially developed for RP, although it has been
272 demonstrated that they can also be run under HILIC conditions with satisfactory results
273 [26]. For this reason, in this work, the performance of the PEG column was studied
274 under both separation modes, as RP × RP has also previously shown relatively good
275 performance in phenolic compounds analysis [13,17]. An independent optimization of
276 the separation conditions was performed for each column, keeping in mind the basic
277 requirements imposed by the 2D set-up used. This LC × LC set-up is based on the use
278 two identical volume sampling loops installed in the switching valve in order to allow
279 the continuous collection and injection of ^{1}D effluent on the ^{2}D. Hence, separations as
280 slow as possible in the ^{1}D are preferred (from 10 to 100 μL min^{-1}, typically) while very
281 fast separations are needed to perform quick ^{2}D separations (3-4 mL min^{-1}) and to
282 maintain the modulation time (and transfer volume) as short as possible. The use of
283 such low flow rates in the ^{1}D limits, in turn, the morphology of the column. It has been
284 repeatedly reported that microbore and narrow columns can provide with the needed
285 efficiency at low flow rates. The characteristics of the columns tested are shown in
286 Table 1. One of the studied grapevine samples was used as a model, and different
287 mobile phases, gradients and flow rates (from 15 to 25 μL min^{-1}) were tested for each
288 column, including acetonitrile/formic acid, acetonitrile/acetic acid, methanol/water/acid
289 or methanol/ammonium acetate buffer mixtures in different proportions. After careful
290 study of the obtained results, the optimum separation conditions for each studied
291 column are reported in Section 2.3. The best conditions involving the use of the PEG
292 column were found under RP conditions. When operated under HILIC-compatible

293 conditions, the PEG column did not produce satisfactory retention of the studied
294 compounds. In any case, it is worth noting that the internal diameter of the available
295 PEG column (2.1 mm) was wider than those from the other tested columns. This fact
296 implies that the used linear velocity is far from optimal values, which means that the
297 obtained separation could be theoretically further improved, although higher flow rates,
298 which are not practical in this application, would be required. Figure 2 shows typical ^{1}D
299 chromatograms obtained under optimum separation conditions for each column. As can
300 be observed, good peak distributions were obtained with the three tested columns,
301 although the diol column was the only one allowing a separation between stilbenoids
302 and proanthocyanidins. Peak capacity values were calculated for the three optimized
303 separations. Results are given in Table 1. The undersampling correction factor $<\beta>$ was
304 also considered to reduce the theoretical ^1n_c as a result of undersampling (Eq. 2),
305 including the sampling time (t_s) later on applied in LC × LC experiments (see below).
306 As can be observed, the diol column produced higher peak capacity values, followed by
307 the PEG and ZIC-HILIC columns (25, 23 and 19, respectively). However, this value
308 should not be the only one taken into consideration to select the best ^{1}D separation
309 method, as increments in ^{1}D peak capacity do not produce enhancements in the two-
310 dimensional peak capacity beyond a certain point because undersampling get worse as a
311 result of narrower ^{1}D peaks (unless ^1t_G is significantly increased) [27].
312
313 The three columns studied in ^{1}D were then tested in a LC × LC set-up in combination
314 with a short partially porous C_{18} column (50 × 4.6 mm, 2.7 µm). The use of relatively
315 short columns with partially porous materials allows obtaining high efficiency values
316 and fast separations, significantly reducing backpressure compared to sub-2 µm
317 columns. In our application, control of pressure as a result of the ^{2}D separations is of

318 utmost importance, as the available switching valve and DAD are not designed to
319 operate at pressures above 400 bar. As can be deduced from the literature [17], C_{18}
320 columns offer unparalleled retention for most of published applications involving a RP
321 separation in ^{2}D. In spite of this, we also studied the possibility of using a PFP
322 (pentafluorophenyl) stationary phase in ^{2}D, maintaining column morphology, although
323 that column did not provide comparable results (data not shown). For each of the
324 studied set-ups, the ^{2}D separation conditions were independently determined; optimum
325 separation conditions are shown in Section 2.3. Flow rate was always maintained as fast
326 as possible in order to reduce ^{2}D analysis time, although gradients shorter than 1 min
327 did not produced successful separations. On the other hand, higher ^{2}D flow rates were
328 avoided due to increased pressure drop and lack of enough sampling rate in the DAD.
329 For these reason, total ^{2}D analysis times were kept at 1.3 min, in order to allow column
330 re-equilibration for 18 s. Moreover, the transfer volume, determined by the available
331 sampling loop volume was also considered. For the three couplings, two 30 μL loops
332 were employed, which provided higher volume than strictly required according to the
333 ^{1}D flow rate and modulation time employed (Table 1). However, we previously
334 demonstrated that by using this additional space, each fraction being transferred was in
335 practice diluted at the head of the ^{2}D column with ^{2}D initial mobile phase. This dilution
336 effect has been demonstrated to be effective to reduce ^{2}D peak distortion related to
337 solvent incompatibility between dimensions [23], considering that there was a solvent
338 strength mismatch in every LC × LC coupling studied here.
339 The results obtained after the application of each optimized LC × LC set-up are
340 illustrated in Figure 3. To make a quantitative comparison of the separation capabilities
341 of each combination, the number of separated peaks and overall resolution, peak
342 capacity values, as well as orthogonality were considered. Firstly, it is important to note,

343 that although 1.3 min cycles may seem too long, the conditions applied in both
344 dimensions allowed to minimize possible negative effects due to undersampling.
345 Considering ^{1}D peak widths before modulation, sampling times from ^{1}D to ^{2}D were
346 estimated; obtained values in the three studied set-ups were always faster than the
347 recommended rate by Murphy, Schure and Foley [28] (i.e., 4 cuts per peak, thus, 2σ), as
348 it can be observed in Table 1. Theoretical peak capacity values derived from the
349 application of eq. 3 are shown in Table 1. As it can be noted, the set-up involving the
350 use of the diol column provided the highest values ($^{2D}n_c$ = 1408). Moreover, in order to
351 give more realistic values, the practical peak capacity (according to eq. 4) was also
352 calculated. This way, the effects of undersampling are also considered; these deleterious
353 effects are related to the re-mix of already separated compounds in the ^{1}D during the
354 collection of the ^{1}D effluent in the modulator. Although one of the premises of LC × LC
355 is that none of the resolution obtained in the ^{1}D is lost in the ^{2}D, in practice this can
356 never be completely achieved [27]; for this reason, the estimation of peak capacity
357 should include the possible losses of ^{1}D peak capacity related to undersampling. Using
358 this approach, practical peak capacity values of the diol × C_{18}, PEG × C_{18} and ZIC-
359 HILIC × C_{18} set-ups were 1080, 961 and 768, respectively. Still, it is important to keep
360 in mind that these peak capacity values are not the real number of peaks that could be
361 separated along the 2D space because there are areas on the 2D chromatogram where
362 peaks do not appear. To evaluate the 2D separation space coverage, orthogonality
363 degree in each set-up was calculated. This parameter gives a measure of the separation
364 quality and allows the comparison between different 2D approaches. System
365 orthogonality (A_0) was calculated taking into account the spread of each peak along the
366 four imaginary lines that cross the 2D space forming an asterisk, that is Z_1, Z_2 (vertical
367 and horizontal lines) and Z_-, Z_+ (diagonal lines of the asterisk) [20]. The ZIC-HILIC ×

368 C_{18} coupling provided an A_0 of 70%, due to a good spread of the peaks around Z_1 and Z_2
369 lines (97 and 91%, respectively). The PEG × C_{18} set-up possessed an A_0 = 45%. This
370 moderated value is related to the poor spread of peaks around the Z_- and Z_+ lines (42 and
371 60%, respectively) as can be observed in Figure 3B, where a peak clustering occurs on
372 the Z_- axis with a low spread. The best orthogonality degree was achieved with the diol
373 × C_{18} coupling obtaining an A_0 of 78% (Figure 3A) corresponding to a high peak
374 spreading around the four axis (93% Z_1, 95% Z_2, 91% Z_- and 75% Z_+). Interestingly, as
375 expected from theory, those set-ups involving a HILIC × RP coupling (Figures 3A and
376 C) provided with higher orthogonality values than the RP × RP set-up involving the use
377 of the PEG column (Figure 3B), for this application. Considering orthogonality values,
378 corrected peak capacities (eq. 5, $^{2D}n_{c,\ corr}$) attained in the diol × C_{18}, PEG × C_{18} and ZIC-
379 HILIC × C_{18} set-ups were 842, 432 and 538, respectively. The application of this
380 correction factor allows a fairer comparison among set-ups, as the whole coupling is
381 evaluated, not only in terms of each dimension separately but also looking at the 2D
382 separation obtainable once coupled. Consequently, as can be deduced from Figure 3, the
383 best conditions were produced using HILIC × RP using a diol column in the ^{1}D coupled
384 to a C_{18} column in the ^{2}D. Moreover, as it can also be inferred from Figure 3, the best
385 ^{1}D peak distribution along the available analysis time was obtained using the diol
386 column, thus, further justifying the use of the mentioned set-up in the present
387 application.
388

389 **3.4. Characterization of the (poly)phenolic profile of grapevine canes by**
390 **HILIC×RP.**

391 The optimized method was then applied for the characterization of the (poly)phenolic
392 profile of canes of two different grapevine varieties, specifically, *Pinot Noir* and

393 *Cabernet Sauvignon*. The analyzed canes were derived from the pruning of different
394 vineyards. After pruning, the canes were stored at ambient temperature for three
395 months. This period was demonstrated to be useful to promote the synthesis of the
396 bioactives present [6]. The 2D plots of the studied samples under the optimum
397 conditions are shown in Figure 4. In order to characterize the separated components, a
398 MS detector was also hyphenated to the LC × LC instrument. The MS used consisted of
399 an ion trap equipped with an electrospray (ESI) interface working on the negative
400 ionization mode. Although this analyzer provided with useful MS data, this instrument
401 does not provide with high scanning speeds, which are very desirable in LC × LC,
402 considering the fast separations (^{2}D) that are carried out just before detection. Table 2
403 summarizes the tentatively identified compounds in both grapevine cane extracts as well
404 as the corresponding data related to their UV-Vis and MS spectra. As can be observed
405 from this Table and Figure 4, most peaks were detected in both varieties, although some
406 others were uniquely found in just one of them. Among the assigned compounds, two
407 families were mainly present, namely proanthocyanidins and stilbenoids. In general,
408 compounds eluted from the ^{1}D according to increasing degree of polymerization (DP);
409 monomers and smaller oligomers were predominantly found in the first section of the
410 2D plot (first 23 min). These compounds were the most abundant in both samples with
411 higher intensities.
412 Catechin and epicatechin (peaks 5 and 6, respectively) were the only flavan-3-ol
413 monomers detected in the studied samples. These two compounds are the basic
414 components of procyanidins; as can be observed in Table 2, the chemical pattern of
415 procyanidin oligomers in grapevine canes was very complex. Moreover, catechin and
416 epicatechin, together with (epi)gallocatechin, are part of prodelphinidins, the other
417 group of proanthocyanidins found in the studied samples. Several procyanidins with DP

418 2 and DP 3 could be tentatively assigned thanks to their typical molecular ions at *m/z*
419 577, 579 and 865, depending on the type of linkage. These compounds also presented
420 characteristic fragment ions corresponding to retro-Diels–Alder (RDA) fission (-152
421 Da), heterocyclic ring fission (HRF, -126 Da), and quinone methide (QM) fission (-289
422 Da) [29]. Moreover, other mono- and digalloylated dimers and trimers were also found
423 (peaks 42, 62, 66 and 67). An example of the MS and MS/MS spectra of a procyanidin
424 trimer digallate as well as its proposed fragmentation pattern can be observed in Figure
425 S1. The typical fragmentation pattern of these components which was already described
426 for other samples [23] was the key for their identification, including the presence of
427 fragments derived from different fission pathways [29]. It has to be pointed out that
428 procyanidins are extensively present in different grape-related components, such as
429 skins, seeds and even wine [30]. The other type of proanthocyanidins identified in these
430 samples was prodelphinidins. In this case, different compounds containing a DP from 2
431 to 5 could be assigned, having also different degree of galloylation. In Table 2, the
432 tentative monomer composition of each prodelphinidin is included in agreement with
433 the molecular ion and main MS/MS fragments detected. For instance, both
434 prodelphinidin dimers detected (peaks 39 and 41) possessed identical molecular ion at
435 *m/z* 593 ($[M-H]^-$), producing MS/MS fragments revealing the presence of (epi)catechin
436 (*m/z* 289) and (epi)gallocatechin (*m/z* 305) (through QM fission). However, in the case
437 of higher molecular weight components, the chemical variability was more complex.
438 For prodelphinidin trimers, three different structures appeared, formed by: two
439 (epi)catechin moieties and one (epi)gallocatechin (peaks 52, 53, 56 and 58) with *m/z* at
440 881 ($[M-H]^-$); a (epi)catechin unit with two (epi)gallocatechin moieties (peak 61) with
441 *m/z* at 897 ($[M-H]^-$), and; a galloylated trimer (peak 65). Likewise, different tetramers
442 could be described in the samples with different basic structure and degree of

443 galloylation (peaks 68, 70, 71 and 72). Interestingly, some of these components were
444 detected as doubly-charged ions. It is important to remark that this is the first work in
445 which prodelphinidins are described in grapevine canes. In any case, the clarification of
446 prodelphinidin oligomers is sometimes not possible only with the information provided
447 by the MS and MS/MS spectra due to the fact that these complex molecules may
448 present different degrees of galloylation as well as different number of
449 (epi)gallocatechin molecules. This implies that some different oligomers may have the
450 same *m/z* and main MS/MS fragments, making the unequivocal assignment very
451 difficult. This is the case of peak 72 that presents a $[M-2H]^{2-}$ at *m/z* 828.6 and could
452 correspond to a prodelphinidin tetramer trigallate or to a prodelphidin pentamer
453 monogallate. The MS and MS/MS spectra of this peak are shown in FigS1C and D, as
454 well as the tentatively proposed fragmentation pattern of both identification options.
455 The use of a high resolution MS analyzer would potentially improve the attainable
456 results as well as the identification certainty through the acquisition of accurate mass
457 values.
458 The other main group of phenolic compounds in grapevine canes are stilbenoids. As can
459 be observed from Table 2, the chemical composition on these compounds was also very
460 complex, involving a great number of different but closely related chemical structures.
461 These components eluted from the ^{1}D according to their increasing size. The most
462 abundant among them was (*E*)-resveratrol (3,5,4'-trihydroxystilbene, peak 1), which
463 was also the most intense peak in general in both samples. Piceatannol (peak 3) was
464 also present in high amounts. Stilbenes monomers, such as resveratrol and piceatannol,
465 present the same MS/MS fragmentation behavior. The fragmentation occurs in the
466 resorcinol ring, which loses two consecutive C_2H_2O, corresponding to one and two
467 neutral losses of 42 Da, respectively [31]. This way, the fragmentation of resveratrol

468 (peak 1, *m/z* 227) is characterized by the production of fragments at *m/z* 187 and 143.
469 Likewise, the fragmentation of piceatannol (peak 3, *m/z* 243) produced fragments at *m/z*
470 201 and 159. The rest of stilbenoids detected in the grapevine canes samples were
471 formed by more complex structures, with varying degree of polymerization. UV-Vis
472 maxima were also useful to assign the separated components as resveratrol presents a
473 UV absorption maximum at 310 nm, whereas, as the size of stilbenoid oligomers
474 increases, the UV maximum shifts to *ca.* 280-290 nm [32]. The above-commented loss
475 of C_2H_2O under MS/MS fragmentation is also characteristic of stilbenoid oligomers;
476 besides the neutral loss of 42 Da, oligomers may also present typical loses
477 corresponding to 94 Da (C_6H_6O), 106 Da (C_7H_6O) and 110 Da ($C_6H_6O_2$) [31]. For
478 instance, peak 4 (*m/z* 453.7, $[M-H]^-$) was tentatively identified as a resveratrol dimer,
479 being the most important fragments derived from this ion those with *m/z* 411 (loss of 42
480 Da), 359 (loss of 94 Da) and 347 (loss of 106 Da). In the same way, peak 2 was also
481 assigned as a resveratrol dimer. These two compounds were related to viniferin,
482 although an unequivocal identification could not be reached with the available tools.
483 Interestingly, a di-glycosylated derivative of this compound was also found in *Pinot*
484 *Noir* canes (peak 48). This compound, not reported previously in grapevine canes, has
485 been detected in Riesling wine [33]. Viniferin diglycoside was characterized by a
486 molecular ion at *m/z* 777, showing MS/MS fragments corresponding to the loss of one
487 or both glycosidic residues (*m/z* 615 and 454). Moreover, three other dimeric stilbenoid
488 derivatives were also detected (peaks 15, 17 and 18). These possessed an ion at *m/z* 469,
489 which was in agreement of a structure based on the combination of (*E*)-resveratrol and
490 piceatannol. Only one resveratrol trimer was detected (peak 19) in the *Pinot Noir*
491 sample (*m/z* 679, Figure 4A) which contrasts with the detection of 6 different
492 resveratrol tetramers (peaks 22, 23, 26, 27, 29 and 30). All these possessed molecular

493 ions at *m/z* 905 and their structure would be related to hopeaphenol and vitisin [34].
494 Additionally, two other stilbenoid tetramers were detected at *m/z* 923 (peaks 36 and 37);
495 their fragmentation pattern indicated that were related to viniferol E, including an
496 additional hydroxyl group in their structure compared to the other tetramers. Moreover,
497 two bigger oligomers, i.e., a resveratrol hexamer (peak 55) and a resveratrol heptamer
498 (peak 47, Figure S1A), were detected in these samples. The generated fragments
499 corresponding to less polymerized resveratrol derivatives helped to assign these
500 components. This is the first report of the presence of these big oligomers in grapevine
501 canes.
502 Besides these components, other compounds were separated and their MS and MS/MS
503 information collected, although no specific assignment could be obtained (see Table 2).
504 Comparing both samples, quite similar profiles were achieved (Figure 4), being (*E*)-
505 resveratrol, piceatannol and resveratrol dimers the most abundant compounds. Although
506 the precise composition changed between *Pinot Noir* and *Cabernet Sauvignon* canes,
507 from a qualitative point of view all the groups of compounds were similarly represented
508 on both samples. In any case, the variability on the (poly)phenolic composition and
509 content in grapevine canes from different varieties has been already reported [5,34].
510 However, this method allows to obtain the (poly)phenolic profile of these complex
511 materials involving different group of polyphenol oligomers, which gives a clear idea of
512 the satisfactory separation power of the developed HILIC × RP method. Furthermore,
513 this application confirms the good possibilities that grapevine canes may have for
514 valorization and attainment of valuable natural components with potential applications
515 in the food, nutraceutical and cosmetic industries.
516
517

518 **4. CONCLUSIONS.**

519 In this work, a new HILIC × RP-DAD-MS/MS method is developed for the profiling of
520 (poly)phenolic compounds present in grapevine canes from several varieties. By
521 combining a diol column in the ^{1}D with a C_{18} column in the ^{2}D, it is possible to obtain
522 their (poly)phenolic profile in around 80 min. The method has shown extremely good
523 separation capabilities, and is characterized by high effective peak capacity (842) and
524 orthogonality (A_0 = 78%). 81 different components were detected in the samples; most
525 of them could be tentatively assigned using the information provided by the MS and
526 DAD detectors employed. Two main (poly)phenolic groups are represented,
527 proanthocyanidins and stilbenoids. Thanks to this development, some components, such
528 as prodelphinidins as well as some highly polymerized stilbenoids have been described
529 for the first time in grapevine canes. Consequently, the interest of the application of LC
530 × LC-based approaches to study complex natural mixtures has been once more
531 confirmed. From the obtained results, it can be deduced that *Vitis vinifera* L. canes have
532 a great potential to be used as an underexploited natural source of bioactive compounds,
533 with potential applications in different fields. The developed methodology might also be
534 a very effective tool to better understand the ongoing mechanisms in grapevine canes
535 triggering the significant increase of the concentrations of some stilbenoids after
536 pruning and during cane storage, thanks to its improved separation capabilities.

537

538

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645

646

647 **FIGURE LEGENDS.**

648 **Figure 1.** Chemical structure of some representative polyphenols present in grapevine
649 (*Vitis vinifera* L.) canes. A) Resveratrol tetramer (Vitisin A); B) Procyanidin trimer
650 digallate; C) Prodelphinidin tetramer (3(E)C-(E)GC).

651

652 **Figure 2.** First dimension chromatograms (280 nm) corresponding to the separation of
653 the polyphenols found in a grapevine cane extract under optimum conditions for each
654 column. For separation conditions, see section 2.3.

655

656 **Figure 3.** Two-dimensional plots and orthogonality values (A_0) obtained using each first
657 dimension column studied (A, diol; B, PEG; C, ZIC-HILIC) coupled to the partially
658 porous C_{18} column in the second dimension under optimized conditions. Dotted lines
659 define area occupied by peaks. For detailed separation conditions, see section 2.3.

660

661 **Figure 4.** Two-dimensional HILIC × RP plots (280 nm) corresponding to the
662 (poly)phenolic profile of Pinot Noir (A) and Cabernet Sauvignon (B) grapevine canes
663 under optimum separation conditions. For peak identification, see Table 2. For detailed
664 separation conditions, see section 2.3.

665

Table 1. Comprehensive two-dimensional method parameters applied to the profiling of (poly)phenolic compounds from grapevine canes.

		Diol × C_{18}	PEG × C_{18}	ZIC-HILIC × C_{18}
^{1}D	L (mm)	150	150	150
	I.D. (mm)	1.0	2.1	1.0
	Particle size (µm)	5	5	3.5
	Flow rate (µLmin^{-1})	18	20	15
	$\overline{w}$ (min)	3.01	3.60	3.40
	$^{1}n_c$	32	27	23
	<β>	1.28	1.20	1.22
	$^{1}n_c$ corr.	25	23	19
^{2}D	$\overline{w}$ (s)	1.40	1.40	1.49
	$^{2}n_c$	44	44	41
LC × LC	Analysis time (min)	92	92	75
	t_s	1.73σ	1.44σ	1.52σ
	Modulation time (min)	1.3	1.3	1.3
	$^{2}V_{inj}$ (V ^{1}D effluent)	30 µL (23.4 µL)	30 µL (26 µL)	30 µL (19.5 µL)
	Z_1	0.93	0.85	0.97
	Z_2	0.95	0.91	0.91
	Z_-	0.91	0.42	0.72
	Z_+	0.75	0.60	0.77
	A_0	78%	45%	70%
	$^{2D}n_c$ theoretical	1408	1188	943
	$^{2D}n_c$ practical	1080	961	768
	$^{2D}n_c$ corr.	842	432	538

<β>, average ^{1}D broadening factor; $^{1}n_c$ corr.: calculated according to eq. 2; t_s, sampling time; A_0, orthogonality; $^{2D}n_{c,\ theoretical}$: $^{1}n_c \times {}^{2}n_c$; $^{2D}n_{c,\ practical}$: calculated according to eq. 4; $^{2D}n_c$ corr.: $^{2D}n_{c,\ practical} \times A_0$

671 **Table 2**. Main polyphenols detected in the grapevine canes samples using the
672 optimized HILIC × RP-DAD-MS/MS method. (E)C, (epi)catechin; (E)gC,
673 (epi)gallocatechin; (E)gCG, (epi)gallocatechin gallate.

Peak	Total t_R (min)	t_R ^{2}D (s)	[M-H]$^-$	λ max (nm)	Main MS/MS fragments	Identification proposed
1	10.03	55.80	227.2	310	210, 186, 159, 143	(*E*)-Resveratrol
2	10.11	60.60	453.0	326	435, 361, 349, 239, 228	Resveratrol dimer
3	11.26	51.50	243.4	324	225, 201, 175, 159	(*E*)-Piceatannol
4	12.70	60.05	453.7	324	435, 411, 359, 347, 339, 253	Resveratrol dimer
5	13.70	41.70	289.7	280	245, 205, 165, 125	Catechin
6	13.73	43.90	289.2	279	245, 205, 125	Epicatechin
7	15.19	53.20	523.3		503, 485, 475, 358, 243	n.i.
8	15.31	60.40	523.2	324	521, 503, 485, 475, 243	n.i.
9	16.27	40.05	433.7	265	385, 223, 205, 179, 153	n.i.
10	16.40	47.75	533.5	310		n.i.
11	16.42	48.85	475.0	268	441, 429, 379, 351, 257	n.i.
12	16.43	49.95	508.4	268	463, 441, 349, 193	n.i.
13	16.51	54.80	521.1	281	485, 475, 387, 357	n.i.
14	16.54	56.35	559.6	310	516, 485, 470, 441, 289	n.i.
15	16.54	56.55	469.3	301	455, 433, 377, 365	Stilbenoid dimer
16	18.99	47.50	475.1	284	454, 377, 349, 255	n.i.
17	19.03	49.65	469.4	301	452, 376, 364, 349, 255	Stilbenoid dimer
18	19.05	50.70	469.4	303	453, 432, 418, 255	Stilbenoid dimer
19	19.23	61.65	679.4	292, 320	661, 586, 452, 345, 257	Resveratrol trimer
20	21.62	48.85	444.6	273	402, 301, 291, 285	n.i.
21	21.81	60.70	695.2	296, 325	601, 575, 467, 453, 241	n.i.
22	29.57	58.10	906.5	284		Resveratrol tetramer
23	29.67	64.30	906.0	284, 325		Resveratrol tetramer
24	33.25	45.00	579.9	280	561, 531, 453, 289, 246	Procyanidin dimer
25	33.30	48.15	549.4		531, 505, 463, 375	n.i.
26	34.66	57.75	906.3	285		Resveratrol tetramer
27	40.02	61.35	905.9	283		Resveratrol tetramer
28	41.05	45.05	577.6	280	559, 451, 425, 407, 289	Procyanidin dimer
29	42.57	58.00	905.8	285		Resveratrol tetramer
30	42.63	61.45	905.8	284, 326		Resveratrol tetramer
31	46.16	39.30	577.7	279	559, 469, 451, 425, 290	Procyanidin dimer
32	46.18	40.80	577.7	280	559, 469, 451, 425, 408, 289	Procyanidin dimer
33	46.21	42.45	577.6	278	559, 469, 452, 426, 333	Procyanidin dimer
34	46.24	44.15	577.7	281	559, 469, 452, 426, 332, 290	Procyanidin dimer
35	46.46	57.25	579.7	282	559, 469, 452, 425, 289	Procyanidin dimer
36	46.47	57.95	923.4	282	903, 827, 693, 479, 469	Stilbenoid tetramer
37	49.04	56.55	923.8	283	903, 829, 693, 469	Stilbenoid tetramer
38	51.48	46.85	757.1	281	605, 405, 230	n.i.

39	53.96	39.50	593.6		575, 465, 453, 439, 407, 305, 289	Prodelphinidin dimer
40	54.20	53.90	939.8		906, 840, 746, 645	n.i.
41	55.28	40.75	593.4		575, 465, 407,305, 289	Prodelphinidin dimer
42	55.36	45.25	729.3	278	665, 603, 577, 559, 441, 407, 289	Procyanidin dimer monogallate
43	55.52	55.05	839.0	285	821, 679, 532	n.i.
44	55.67	64.25	1045.1	286, 325	1027, 988, 758, 602	n.i.
45	56.63	43.65	745.3	278	645, 592, 453, 341	Procyanidin dimer
46	56.79	53.55	839.8	285	820, 679, 593, 532	n.i.
47	58.22	61.40	790.9*	286	1355, 1131, 906, 792, 679, 451	Resveratrol heptamer
48	59.44	56.25	777.6	288	615, 454	Viniferin diglycoside
49	59.52	61.20	781.8*	288	1439, 1351, 1040, 949, 887, 688	n.i.
50	60.49	41.35	865.6	279	847, 739, 713, 695, 577	Procyanidin trimer
51	60.52	43.35	865.8	278	848, 821, 801, 663, 591, 518, 475	Procyanidin trimer
52	60.54	44.30	881.3	278	729, 711, 591, 577, 559, 439	Prodelphinidin trimer (2(E)C--(E)gC) // Procyanidin dimer digallate
53	60.58	47.15	881.5	280	729, 711, 591, 577, 559, 439	Prodelphinidin trimer (2(E)C--(E)gC) // Procyanidin dimer digallate
54	60.63	49.95	897.7	325	877, 801, 725, 605, 589, 578	Dp-3-p-coumaroilglucoside-(epi)catechin
55	60.74	56.25	1359.7	285	1265, 1253, 1131, 906, 813	Resveratrol hexamer
56	61.87	46.00	881.5	281	861, 753, 727, 709, 791, 547	Prodelphinidin trimer (2(E)C--(E)gC) // Procyanidin dimer digallate
57	61.97	52.00	1195.7	282	1043, 905, 707	Resveratrol tetramer + Catechin
58	64.26	33.75	881.3	278	863, 755, 729, 711, 593, 575, 287	Prodelphinidin trimer (2(E)C--(E)gC) // Procyanidin dimer digallate
59	64.50	47.80	1027.6	279	905, 782, 724, 659, 575, 313	
60	64.70	59.90	1175.1		1137, 1027, 944, 843, 729, 592, 493, 381	n.i.
61	65.71	42.60	897.9	280	838, 769, 743, 727, 607, 591, 467, 303	Prodelphinidin trimer ((E)C+ 2(E)gC)
62	65.74	44.25	1017.0	280	999, 955, 891, 866, 847, 740, 729, 696, 678, 602, 559, 451, 407, 289	Procyanidin trimer monogallate
63	65.76	45.35	1015.2	280	997, 967, 851, 789, 713, 610, 427	n.i.
64	66.01	60.60	922.7*	286	1811, 1555, 905, 875 827, 799	n.i.
65	69.53	37.95	1035.2		1015, 907, 881, 863, 847, 755, 745, 729	Prodelphinidin trimer monogallate ((E)C--(E)gCG or (E)CG--(E)C--(E)gC)
66	69.65	44.65	1169.6	280	1151, 1043, 1017, 999, 881, 865, 847, 729, 577	Procyanidin trimer digallate
67	70.90	42.05	1167.7	279	1152, 1017, 999, 877, 865, 742, 729, 591	Procyanidin trimer digallate
68	76.06	39.70	1171.8			Prodelphinidin tetramer (3 (E)C--(E)gC)
69	76.14	44.10	751.9*	278	1377, 1103, 989, 664, 487	n.i.

70	77.30	36.15	1186.5	290		Prodelphinidin tetramer (2 (E)C--2(E)gC)
71	78.71	42.80	735.0*	279	1443, 1339, 1154, 1017, 865, 578, 289	Prodelphinidin tetreamer digallate (2(E)CG--(E)C--(E)gC or (E)CG--2(E)C--(E)gCG)
72	78.75	45.20	828.6*	282	1492, 1370, 1016, 865, 745, 571	Prodelphinidin tetramer trigallate ((E)CG--2(E)gCG--(E)gC or (E)C--3(E)gCG // Prodelphidin pentamer monogallate ((E)CG--4(E)gC or (E)C--(E)gCG--3(E)gC)
73	81.34	44.45	917.9*	282	1541, 1487, 1087, 1029, 841, 576	n.i.
74	82.66	45.55	884.3*	280	1568, 1483, 1316, 1192, 1065, 739, 591	n.i.
75	82.67	46.05	894.9*	281	1618, 1375, 1316, 1179, 816, 603	n.i.
76	83.92	43.30	881.4*	281	1469, 1183, 806, 795, 728, 590, 577, 289	Procyanidin-related
77	83.96	45.60	886.0*	280	1579, 1483, 1354, 1179, 995, 865, 808, 741, 577	Procyanidin-related
78	86.54	44.25	1163.7	280		n.i.
79	86.57	46.30	1171.7	279		n.i.
80	89.16	45.35	1163.5	278		n.i.
81	94.24	38.10	1028.9	278		n.i.

674 n.i., Not identified; *ions detected as $[M-2H]^{2-}$

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