Review Article

Recent Applications of Mass Spectrometry in the Study of Grape and Wine Polyphenols

Riccardo Flamini

*Consiglio per la Ricerca e la Sperimentazione in Agricoltura-Centro di Ricerca per la Viticoltura (CRA-VIT),
Viale XXVIII Aprile 26, 31015 Conegliano, Italy*

Correspondence should be addressed to Riccardo Flamini; riccardo.flamini@entecra.it

Received 24 September 2012; Accepted 12 October 2012

Academic Editors: D.-A. Guo, T. Stafilov, and M. Valko

Copyright © 2013 Riccardo Flamini. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Polyphenols are the principal compounds associated with health benefic effects of wine consumption and in general are characterized by antioxidant activities. Mass spectrometry is shown to play a very important role in the research of polyphenols in grape and wine and for the quality control of products. The soft ionization of LC/MS makes these techniques suitable to study the structures of polyphenols and anthocyanins in grape extracts and to characterize polyphenolic derivatives formed in wines and correlated to the sensorial characteristics of the product. The coupling of the several MS techniques presented here is shown to be highly effective in structural characterization of the large number of low and high molecular weight polyphenols in grape and wine and also can be highly effective in the study of grape metabolomics.

1. Principal Polyphenols of Grape and Wine

Polyphenols are the principal compounds associated to health benefic effects of wine consumption. A French epidemiological study performed in the end of 1970s reported that in France, despite the high consumption of foods rich in saturated fatty acids, the incidence of mortality from cardiovascular diseases was lower than that in other comparable countries. This phenomenon was called “the French paradox” and was related to the beneficial effects of red wine consumption [1]. In general, polyphenols have antioxidant activities. Their activity as peroxy radical scavengers and in the formation of complexes with metals (Cu, Fe, etc.) has been shown by *in vitro* studies. Moreover, the ability of polyphenols to cross the intestinal wall of mammals confers their biological properties.

Flavan-3-ols are one of the principal classes of grape polyphenols which include (+)-catechin and (–)-epicatechin, and their oligomers called procyanidins, proanthocyanidins, and prodelphinidins. B-type and A-type procyanidins and proanthocyanidins (the latter are condensed tannins) are present in the grape skin and seeds; tannins are mainly present in seeds, and prodelphinidins are polymeric tannins composed of gallocatechin units (structures in Figure 1).

During winemaking the condensed (or nonhydrolyzable) tannins are transferred to the wine and contribute strongly to the sensorial characteristic of the product. In the mouth, the formation of complexes between tannins and saliva proteins confers to the wine the sensorial characteristic of astringency: bitterness and astringency of wine is linked to tannins structure, in particular galloylation degree (DG) and polymerization degree (DP) of flavan-3-ols [2, 3]. Grape tannins are used as active ingredients in medicinal products characterized by antioxidant plasma activity and for the treatment of circulatory disorders (capillary fragility, microangiopathy of the retina, reducing of platelet aggregation, decreasing of the susceptibility of healthy cells towards toxic and carcinogenic agents, and antioxidant activity toward human low density lipoprotein) (see [4], and references cited herein).

Flavonols are another important class of grape polyphenols. These compounds are mainly present in the skin of berry; the principal are quercetin, kaempferol, and myricetin present in glycoside forms such as glucoside, glucuronide, and rutin. Recently, also isorhamnetin, laricitrin, and syringetin were identified in grape [5, 6]. The structures of flavonols are reported in Figure 2(b). The main biological activity of quercetin is to block human platelets aggregation, and it seems that it inhibits carcinogens and the cancer

FIGURE 1: B-type and A-type flavan-3-ol dimers and trimers present in the grape seeds.

FIGURE 2: (a) The principal monomer anthocyanins of grape: the glucose residue can be linked to an acetyl, coumaroyl, or caffeoyl group. (b) The principal flavonols of grape.

cell growth in human tumors (see [4] and references cited herein).

Stilbene compounds are the principal phytoalexins of grape: they include *cis*- and *trans*-resveratrol (3,5,4'-trihydroxystilbene) and their glucoside derivatives (*cis*- and *trans*-piceid), piceatannol (3,4,3',5'-tetrahydroxy-*trans*-stilbene), and stilbene oligomers (viniferins). A number of studies evidenced anticancer, cardioprotection, anti-inflammatory and antioxidant activities, and platelet aggregation inhibition of *trans*-resveratrol [7–13]. Grapevine synthesizes viniferins in different parts of the plant (roots, clusters, and stems) in particular ϵ -viniferin, two δ -viniferin glucosides, and pallidol [14, 15]. Moreover, viniferins can arise from the oligomerisation of *trans*-resveratrol in grape tissues as active defense of the plant against exogenous attacks or could be produced from resveratrol by extracellular enzymes released from the pathogen in an attempt to eliminate undesirable toxic compounds [16, 17]. Structures of the principal vine viniferins are showed in Figure 3.

Anthocyanins are the compounds responsible for the red color of grapes and wines. Principal anthocyanins of *Vitis vinifera* varieties are delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv), present in the skins as 3-O-monoglucoside, 3-O-acetylmonoglucoside, and 3-O-(6-O-*p*-coumaroyl)monoglucoside. Often, also Mv-3-O-(6-O-*p*-coumaroyl)monoglucoside is present. More recently, pelargonidin (Pg) 3-O-monoglucoside was found in grape [24]. Often the not *V. vinifera* (hybrid) red grapes also contain diglucoside anthocyanins with the second glucose molecule linked to the C-5 hydroxyl group (structures showed in Figure 2(a)).

The anthocyanin profile is also determined for the study of grape chemotaxonomy; for example, the presence of 3,5-O-diglucoside anthocyanins is used to distinguish between *V. vinifera* and hybrid grape varieties, the former being characterized by low presence or practical absence of these compounds. Moreover, grape anthocyanins are antioxidant and natural colorants used in the nutraceutical, food, and pharmaceutical industries [26–28].

During the wine aging, anthocyanins are undergone reactions with other matrix compounds, and new molecules with different chromatic characteristics, with respect to their precursors, are formed [29]. As a consequence, the anthocyanic profile of wine changes dramatically during aging; for example, the LC-chromatogram of a 4-month aged wine recorded at 520 nm shows as main signals the grape anthocyanins, and after 2-year aging, these signals disappear completely, and a broad peak due to the new anthocyanin derivatives overlaps the latter part of the chromatogram [30, 31]. Reaction of anthocyanins with flavan-3-ols, procyanidins, and tannins shifts the wine from purple-red to brick-red hue, and the formation of pyranoanthocyanins, stable structures formed by reaction between anthocyanins and acetaldehyde, pyruvic acid, vinylphenol, vinylcatechol, vinylguaiacol, or vinyl(epi)catechin, toward orange hue [20, 21, 32–35].

More than hundred structures belonging the pigment families of anthocyanins, pyranoanthocyanins, direct flavanol-anthocyanin condensation products and acetaldehyde-mediated flavanol-anthocyanin condensation products (anthocyanin linked to flavan-3-ol either directly or by ethyl bridge), were identified in red wines. Structures of principal anthocyanin-derivatives are showed in Figure 4, [30].

FIGURE 3: Continued.

FIGURE 3: *trans*-resveratrol (1) and principal viniferins of the vine: *Z*- ϵ -viniferin (2), *E*- ϵ -viniferin (3), *E*- ω -viniferin (4), *Z*- ω -viniferin (5), pallidol (6), *E*-ampelopsin D (7), *E*-quadrangularin A (8), α -viniferin (9), *E*-*cis*-miyabenol C (10), *Z*-miyabenol C (11), *E*-miyabenol C (12), isohopeaphenol (13), ampelopsin H (14), vaticanol C isomer (15), and hopeaphenol (16).

TABLE 1: LC retention times (RTs), maximum adsorption UV-Vis wavelengths, and mass spectra data of flavonols identified in *Petit Verdot* grape skins.

Flavonol	HPLC RT (min)	$\lambda_{\max}$ (nm)	$[M - H]^+$ and product ion (m/z)
Myricetin-3-glucuronide	13.9	257(sh), 261, 301(sh), 353	495, 319
Myricetin-3-glucoside	14.5	257(sh), 262, 298(sh), 355	481, 319
Quercetin-3-glucuronide	18.0	257, 265(sh), 299(sh), 354	479, 303
Quercetin-3-glucoside	18.8	256, 265(sh), 295(sh), 354	465, 303
Laricitrin-3-glucoside	19.9	256, 265(sh), 301(sh), 357	495, 333
Kaempferol-3-glucoside	22.6	265, 298(sh), 320(sh), 348	449, 287
Isorhamnetin-3-glucoside	24.3	255, 265(sh), 297(sh), 354	479, 317
Syringetin-3-glucoside	24.9	255, 265(sh), 300(sh), 357	509, 347
Laricitrin-3-galactoside	19.4	256, 265(sh), 302(sh), 357	495, 333
Kaempferol-3-galactoside	21.1	266, 292(sh), 320(sh), 348	449, 287
Kaempferol-3-glucuronide	21.9	265, 290(sh), 320(sh), 348	463, 287
Quercetin-3-(6-acetyl)glucoside	22.9	257, 265(sh), 295(sh), 352	517, 303
Syringetin-3-(6-acetyl)glucoside	30.4	255, 265(sh), 298(sh), 358	517, 347

2. Liquid Chromatography/Mass Spectrometry Analysis of Nonanthocyanic Polyphenols in Grape and Wine

Liquid Chromatography/Mass Spectrometry (LC/MS) coupled with Multiple Mass Spectrometry (MS/MS and MSⁿ) is the most effective tool for the structural characterization of low molecular weight (MW) polyphenols in grape extracts and wine. It is also widely used to characterize high-MW compounds, such as procyanidins, proanthocyanidins, prodelphinidins, and tannins [37–40]. In general, these methods require minor sample purification, and MS/MS allows characterization of both aglycone and sugar moiety.

A study of flavonols in different *V. vinifera* red grape extracts showed, in addition to myricetin and quercetin 3-O-glucosides and 3-glucuronides, and to kaempferol and isorhamnetin 3-O-glucosides, the presence of laricitrin and syringetin 3-glucosides. Also, minor flavonols, such as kaempferol and laricitrin 3-galactosides, kaempferol-3-glucuronide, and quercetin and syringetin 3-(6-acetyl)glucoside, were identified [5]. Table 1 reports the flavonols identified in

Petit Verdot grape skins extract. Extraction was performed by using a methanol/H₂O/formic acid 50:48.5:1.5 (v/v/v), and the analytes were separated from anthocyanins by performing solid-phase extraction (SPE) using a combined reverse-phase and cationic-exchanger commercial cartridge. After sample loading, the cartridge was washed with HCl 0.1 M solution, and the flavonol fraction containing neutral and acidic polyphenols was recovered with methanol. LC analysis was performed by using a reverse-phase C₁₈ column with elution gradient with water/acetonitrile/formic acid 87:3:10 v/v/v and 40:50:10 v/v/v. Flavonols were detected by performing analysis with the mass spectrometer operating in positive ion mode.

Usually, LC/MS analysis of resveratrol (3,5,4'-trihydroxystilbene) and piceatannol (3,4,3',5'-tetrahydroxy *trans*-stilbene) in grape is performed in negative-ion mode. Chromatographic separation can be performed by using a reverse-phase C₁₈ column and elution gradient program with a binary solvent composed of water/0.1% formic acid and methanol (e.g., 33% methanol for 40 min, 33% → 100% methanol in 15 min, 100% methanol for 5 min) [42].

FIGURE 4: Above: compounds formed in wines during aging: (a) structure with direct linkage between anthocyanin and flavan-3-ol proposed by Somers [18] and (b) anthocyanin-flavan-3-ol structure by ethyl bridge proposed by Timberlake and Bridle [19]. Below: structures of C-4 substituted anthocyanins identified in aged red wines formed by reaction with pyruvic acid, vinylphenol, vinylcatechol, vinylguaiacol, and vinyl(epi)catechin [20–23].

FIGURE 5: Product negative-ion spectrum of direct infusion ESI-generated $[M-H]^-$ species of *trans*-resveratrol (a) and piceatannol (b) [25].

FIGURE 6: Collisionally induced fragmentation patterns of $[M-H]^-$ ions of *trans*-resveratrol at m/z 227 (compound 1) considering that the deprotonation reaction occurred on the phenol moiety (Scheme 1a), and of $[M-H]^-$ ions of *trans*-resveratrol (R = H, compound 1) and piceatannol at m/z 243 (R = OH, compound 2) considering that the deprotonation reaction occurred on the resorcinol moiety (Scheme 1b), [25].

Recently, the mechanisms of the fragmentation of *trans*-resveratrol and piceatannol were studied by MS^n and deuterium exchange experiments and performing accurate mass measurements [25]. The product ion spectra of *trans*-resveratrol $[M-H]^-$ ion at m/z 227 and of piceatannol at m/z 243 are reported in Figure 5. Fragmentation patterns of

the two compounds are reported in Figure 6. Fragmentations were confirmed by deuterium labeling experiments by dissolving the standard compounds in deuterated methanol: the deprotonated molecules of *trans*-resveratrol and piceatannol were shifted at m/z 229 and m/z 246, respectively, proving the occurrence of OH hydrogen exchanges.

FIGURE 7: Fragmentation patterns of m/z 867 trimeric procyanidins studied in ESI-MS positive-ion mode [36].

Extraction of proanthocyanidins (PAs) and tannins from seeds and skins can be performed from the powder produced by grinding frozen material using a methanol/ H_2O 25:75 (v/v) solution (e.g., three consecutive extractions for 15 min under stirring at room temperature using ultrasounds [44, 45] or with an acetone/ H_2O 60:40 (v/v) solution [3]. After removing of organic solvent, the aqueous residue has to be washed with hexane in order to eliminate lipophilic substances. The extract can be then fractionated on a column for gel filtration of natural products Sephadex LH-20 by eluting different fractions with ethanol and acetone aqueous solutions [44, 45]. Alternatively, purification of aqueous extract can be done by performing chromatography on a methacrylic size-exclusion resin and elution from the column of two fractions with ethanol/ H_2O /TFA 55:45:0.02 v/v/v and acetone/ H_2O 30:70 v/v [3]. The two solutions are pooled, concentrated under vacuum, and freeze dried. Further purification of the residue can be performed on divinylbenzene-polystyrene resin: flavan-3-ol monomers are recovered with water and ether, then PAs with polymerization degree of 3 units (DP3) with methanol. Finally, the fraction containing DP10 is recovered with acetone/ H_2O 60:40 v/v. Seed extract, or grape juice, can be also purified by SPE using a reverse-phase C_{18} cartridge. Extract is suspended

in water, and the solution is passed through the cartridge previously conditioned by passage of methanol and water, and after sample loading, the cartridge is rinsed with water, and the fraction containing PAs is eluted with acetone/ H_2O /acetic acid 70:29.5:0.5 v/v/v [46, 47].

Extraction of tannins from skins is performed by preliminary removing the low MW phenolics (in particular anthocyanins) immersing the skins in a 12% v/v ethanol solution for 72 h at 4°C. Then, skins are ground in methanol, and the solution is kept in immersion for other two hours at 4°C. Solid parts are extracted again with acetone/ H_2O 60:40 v/v at 4°C overnight. The two extracts are concentrated under vacuum and fractionated separately on a size-exclusion resin. After removing of sugars and phenolic acids by washing the column with ethanol/ H_2O /TFA 55:45:0.02 (v/v/v) and acetone/ H_2O 30:70 (v/v), the fraction containing PAs with DP 12–20 is recovered with acetone/ H_2O 60:40 v/v [3].

LC/ESI-MS analysis of PAs is usually performed by reverse-phase chromatography, even if normal phase chromatography using silica columns provided a satisfactory separation of oligomers based on their MW [46, 47]. The LC/ESI-MS positive-ion chromatogram of a grape seeds extract (analysis performed by a reverse-phase column and gradient elution with a binary solvent composed of aqueous

FIGURE 8: Fragmentation pathways of monomer catechin in positive-ion mode: retro-Diels-Alder fission (RDA), heterocyclic ring fission (HRF), benzofuran forming fission (BFF), and loss of water molecule [41].

0.1% formic acid and acetonitrile/0.1% formic acid) shows the signals of protonated catechin and epicatechin at m/z 291, protonated catechin/epicatechin gallate at m/z 443, protonated catechin/epicatechin dimer at m/z 579, and protonated catechin/epicatechin gallate dimers and trimers at m/z 731, m/z 883 and m/z 867, respectively [48].

PAs were also characterized by direct-infusion ESI-MS without performing chromatographic separation. Negative-ion analysis of the extract dissolved in methanol/acetonitrile showed the highest intensity of ions including multiplied charged species [50]. Moreover, simpler mass spectra were recorded due to the absence of intense adduct ion species and to the production of more multiply charged ions with respect to the positive ionization mode. The $[M-H]^-$ and $[M-2H]^{2-}$ species of PAs with DP3 and DP9 are reported in Table 2. Abundant $[M-H]^-$ singly charged ions separated by 288 Da in the ranges m/z 289–2017 and m/z 441–1881, were observed, corresponding to procyanidins (PCs) with DP 1–7 and procyanidin monogallates (PC1Gs) with DP 1–6, respectively. PAs with DP9 show the additional larger $[M-H]^-$

ions derived from PC1G with DP7, PC2Gs (procyanidin digallates) with DP6 and DP7, and PC3Gs (trigallates) with DP4 and DP5.

The fragmentation pathways of $[M-H]^-$ and $[M-3H]^{3-}$ ions at m/z 577, 575, 729, 727, and m/z 441 are probably due to the cleavage of the interflavanic bond, Retro-Diels-Alder (RDA) fission on the C ring followed by the elimination of water with formation of $[M-H-152]^-$ (m/z 713, 425, 865, and 577) and $[M-H-152-H_2O]^-$ (m/z 695, 407, 847, and 559) ions, and $[M-H-126]^-$ ions at m/z 739 and 451 formed by elimination of a phloroglucinol molecule. The ion at m/z 881 corresponds to a epicatechin-gallate dimer or a epicatechin-epicatechin-epigallocatechin trimer (two isobaric compounds). Doubly charged ions showed a series of abundant ions separated by 144 Da from m/z 652.4 to 1948.8, which correspond to the $[M-2H]^{2-}$ ions of PC1Gs with DP 4–13. Increase of the orifice voltage showed two different fragmentation patterns of trimeric species: formation of two ions at m/z 575 and 573 from the ions at m/z 863 (A-type) and formation of ions at m/z 711 due to RDA fragmentation.

TABLE 2: $[M - H]^-$ and $[M - 2H]^{2-}$ ions of proanthocyanidins with degree of polymerization (DP) 3 and 9 (na: not assigned) [50].

[illegible]

FIGURE 9: Positive-ion mode fragmentation pathways of B-type trimer m/z 883: retro-Diels-Alder fission (RDA), heterocyclic ring fission (HRF), benzofuran forming fission (BFF), quinone methide fission (QM), and loss of water molecule. QM_{CD} : the ion derived from the QM fission of ring-C/ring-D linkage bond by the loss of upper unit and QM_{FG} : the ion derived from the QM fission of ring-F/ring-G linkage bond by the loss of lower unit [41].

Neutral loss of 152 Da (corresponding to 3,4-dihydroxy- α -hydroxystyrene) induces formation of two fragments at m/z 285 and 289 generated by cleavage of the A-type interflavanic linkage [51].

Operating in positive ionization mode provides also the signals of PCs and PAs protonated molecules: $[M+H]^+$ ion of catechin dimers, trimers, and tetramers at m/z 579, 867, and 1155, of their mono- and digalloyl derivatives at m/z 731, 883, 1019, 1171, 1307, and 1459, of trimers and tetramers trigalloyl derivatives at m/z 1323 and 1611, together with those of $[M+H]^+$ ions of flavan-3-ols pentamers, hexamers, and heptamers (m/z 1443, 1731, 2019), their monogalloyl derivatives (m/z 1595, 1883, 2171), pentamers and hexamers digalloyl derivatives (m/z 1747 and 2035), and pentamers and hexamers trigalloyl derivatives (m/z 1899 and 2187) are observed [45].

In another study, the presence of galloylated A-type procyanidins in grape seeds was evidenced [52]. Procyanidins were extracted from seeds with methanol and fractionated

using graded methanol/chloroform precipitation in order to obtain the oligomers with lower MW. ESI-MS spectra recorded in positive mode showed the presence of $[M+H]^+$ ions of A-type and B-type nongalloylated and monogalloylated procyanidins with DP 2–5, and of digalloylated oligomers with DP 2–3. A-type procyanidins occurred with abundance 60%–80% with respect to the corresponding type-B species, the abundance of monogalloylated dimers was 20% of the abundance of the corresponding nongalloylated ones. For higher DP, the abundance of nongalloylated oligomers was higher of the corresponding galloylated oligomers. The type-A interflavanic linkages were present in the terminal units, whereas the type-B interflavanic linkages were extension units.

Figure 7 shows the positive-ion MS³ fragmentations of the most intense trimeric procyanidins signals in the spectra at m/z 867 [36]. The schemes of positive fragmentation patterns for monomer catechin and of a B-type trimer at m/z 883 are reported in Figures 8 and 9, respectively [41]. Table 3

FIGURE 10: Fractionation of polyphenols in red wine (PA, proanthocyanidins) [43].

reports the positive-ion LC/ESI-MSⁿ product ions of flavan-3-ol monomers and PAs dimers, trimers, and oligomers.

A recent study reports the identification of fourteen flavan-3-ol monoglycosides in *Merlot* grape seeds and wine extracts [53]. Analyses were performed using an ESI-quadrupole time of flight mass spectrometry system operating in negative-ion mode. Compounds were identified on the basis of their exact masses and specific fragmentation patterns and as aglycones showed (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, and epicatechin gallate monomeric units.

Several methods of sample preparation were proposed to perform analysis of polyphenols in wine. The nonanthocyanic polyphenols identified in four different wine varieties (*Tempranillo*, *Graciano*, *Cabernet Sauvignon*, and *Merlot*) by performing LC/ESI-MS analysis are reported in Table 4. The analytes were extracted from 50 mL of wine previously reduced to 15 mL under vacuum in order to eliminate ethanol. Two consecutive extractions were performed by using diethyl ether and ethyl acetate. The two organic phases were combined, the solvent removed, and the residue was dissolved in a methanol/H₂O solution. LC analyses were

performed using a reverse-phase C₁₈ column performing the gradient elution with a binary solvent composed of H₂O/acetic acid 98:2 v/v and H₂O/acetonitrile/acetic acid 78:20:2 v/v/v [54]. The compounds were identified on the basis of their fragment ions and maximum absorption wavelengths recorded in the mass and UV-Vis spectra, respectively. Table 4 reports a number of compounds included in different classes of phenols, such as flavonols, hydroxycinnamoyltartaric acids, stilbene compounds (*cis*- and *trans*-resveratrol, piceid), phenolic acids, flavan-3-ols, and dimeric (B1, B3, B4, and B5) and trimeric (C1, T2 and T3) procyanidins.

Two different purification methods by using size-exclusion and reverse-phase chromatography were used to perform sample preparation for the analysis of PCs and PAs in wine. A volume of dealcoholized wine was passed through a size-exclusion resin, the stationary phase was washed with water, and the fraction containing simple polyphenols was eluted with ethanol/H₂O/TFA 55:45:0.005 v/v/v. The fraction containing dimers and trimers was recovered with acetone/H₂O 60:40 v/v [55]. Sample preparation by reverse-phase chromatography was instead performed using

FIGURE 11: Profile of anthocyanins in *Clinton* skin extract recorded by direct-injection positive-ion ESI/MS.

FIGURE 12: LC-Chip/ESI-QTOF-MS analysis of *Clinton* grape skins extract: (a) total ion chromatogram (TIC); (b) extracted ion chromatogram (EIC) of the signal at m/z 611; (c) EIC of the signal at m/z 625; (d) EIC of the signal at m/z 655 [49].

TABLE 3: LC/ESI-MSⁿ product ions of flavan-3-ol monomers, dimers, and oligomers recorded in positive-ion mode. Neutral losses are shown in parentheses. C, EC, and GC: catechin, epicatechin, and gallo catechin, respectively. (E)C and (E)GC: (epi)catechin and (epi)gallo catechin, respectively. (E) indicates either catechin/epicatechin or gallo catechin/epigallo catechin.

Compound	[M + H] ⁺	HRF _C	RDA _C	BFF _C	BFF _C /H ₂ O	HRF _C /H ₂ O	
C	291	165 (126)	139 (152)	169 (122), 123	151 (140), 123	147 (144)	
EC	291	165 (126)	139 (152)	169 (122), 123	151 (140), 123	147 (144)	
GC	307	181 (126)	139 (168)	169 (138), 139	151 (156), 139	163 (144)	
[M + H] ⁺	QM _{CD†}	HRF _C	RDA _C	RDA _F	HRF _C H ₂ O/BFF _F	RDA _F /HRF _C	
(E)GC-(4,8)-(E)C	305	469 (126)	427 (168)	443 (152)	317 (152)	329 (140)	317 (126)
(E)GC-(4,6)-(E)C		469 (126)	427 (168)	443 (152)	317 (152)	329 (140)	317 (126)
(E)C-(4,8)-(E)C		453 (126)	427 (152)	427 (152)	301 (152)	313 (140)	301 (126)
(E)C-(4,8)-(E)C	289	453 (126)	427 (152)	427 (152)	301 (152)	313 (140)	301 (126)
(E)C-(4,8)-(E)C		453 (126)	427 (152)	427 (152)	301 (152)	313 (140)	301 (126)
(E)C-(4,8)-(E)C	289	453 (126)	427 (152)	427 (152)	301 (152)	313 (140)	301 (126)
[M + H] ⁺	QM _{CD†} /QM _{CD‡}	QM _{FGI} /QM _{FGI}	QM _{CD‡} /RDA _F /HRF _F	HRF _C /QM _{FGI}	HRF _C /RDA _F	HRF _C /H ₂ O/BFF _F	
(E)C-(E)GC-(E)C	883	—/595	593/—	595 (288)/443 (152)/317 (126)	757 (126)/467 (290)	757 (126)/589 (168)	757 (126)/601 (156)
(E)GC-(E)GC-(E)C	899	—/595	609/291	595 (304)/443 (152)/317 (126)	773 (126)/483 (290)	773 (126)/605 (168)	773 (126)/617 (156)
(E)C-(E)C-(E)C	867	—/579	577/291	579 (288)/427 (152)/301 (126)	741 (126)/451 (290)	741 (126)/589 (152)	741 (126)/601 (140)
(E)C-(E)C-(E)C	867	—/579	577/—	579 (288)/427 (152)/301 (126)	741 (126)/451 (290)	741 (126)/589 (152)	741 (126)/601 (140)
(E)C-(E)C-(E)C	867	—/579	577/—	579 (288)/427 (152)/301 (126)	741 (126)/451 (290)	741 (126)/589 (152)	741 (126)/601 (140)
[M + H] ⁺	Diagnostic ions						
(E)C-(E)C-(E)C-(E)C	1155	867, 865, 579					
(E)C-(E)C-(E)GC-(E)C	1171	883, 881, 595					
(E)C-(E)GC-(E)GC-(E)C	1187	899, 897, 595					
(E)GC-(E)GC-(E)GC-(E)C	1203	913, 899, 595					
(E)C-(E)C-(E)C-(E)C	1443	1155, 1153, 867, 579					
(E)C-(E)GC-(E)C-(E)C	1459	1171, 1169, 867, 579					
(E)C-(E)GC-(E)GC-(E)C	1475	1187, 1185, 883, 579					
(E)C-(E)C-(E)C-(E)C-(E)C	1731	1441, 1143, 1155, 867, 579					

RDA: retro-Diels-Alder fission; HRF: heterocyclic ring fission; BFF: benzofuran forming fission; QM: quinone methide fission; QM_{CD}†: the ion derived from the QM fission of ring-C/ring-D linkage bond by the loss of upper unit; QM_{CD}‡: the ion derived from the QM fission of ring-C/ring-D linkage bond by the loss of lower unit; QM_{FGI}†: the ion derived from the QM fission of ring-F/ring-G linkage bond by the loss of upper unit; QM_{FGI}‡: the ion derived from the QM fission of ring-F/ring-G linkage bond by the loss of lower unit [41].

TABLE 4: Nonanthocyanin phenolic compounds identified by LC/ESI-MS in wines from *Vitis vinifera* varieties *Tempranillo*, *Graciano*, *Cabernet Sauvignon*, and *Merlot*. Principal mass fragments and maximum UV-Vis absorption wavelengths are reported. (s): shoulder [54].

RT (min)	Compound	(m/z)		$\lambda_{\max}$ (nm)
		$[M - H]^-$	Fragments	
7.6	Gallic acid	169	125	272
14.0	Protocatechuic acid	153	109	294, 260
15.7	Dihydroxyphenylethanol	153		280
16.7	<i>trans</i> -caffeoyltartaric acid	311	179	330, 298(s)
19.2	2,3-Dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one	211		310, 280
19.5	Methyl gallate	183	169, 125	272
20.5	Tyrosol	137		275
22.3	(epi)gallocatechin-(epi)catechin dimer	593	425	276
22.7	Procyanidin B3	577	425, 289	280
23.0	Procyanidin B1	577	425, 289	280
24.2	<i>trans</i> -coumaroyl-tartaric acid	295	163	313
27.3	(+)-catechin	289		279
28.0	Procyanidin T2	865	713, 577, 289	280
28.3	<i>trans</i> -ferulyltartaric acid	325	193	329, 301(s)
28.7	Hexose ester of vanillic acid	329	167	nd
29.0	Procyanidin T3	865	713, 577, 289	280
29.7	Vanillic acid	167		289, 262
30.5	Procyanidin B4	577	425, 289	283
31.3	<i>trans</i> -caffeic acid	179	135	323
33.0	Hexose ester of <i>trans</i> - <i>p</i> -coumaric acid (1)	325	163, 145	311
33.6	Procyanidin B2	577	425, 289	280
34.6	Syringic acid	197		277
37.0	Hexose ester of <i>trans</i> - <i>p</i> -coumaric acid (2)	325	163, 145	312
38.7	(-)-epicatechin	289		279
40.2	Trimeric procyanidin	865	713, 577, 289	280
41.3	Ethyl gallate	197	169, 125	273
42.2	Procyanidin C1	865	713, 577, 289	282
43.2	<i>trans</i> - <i>p</i> -coumaric acid	163	119	309
43.5	Trimeric procyanidin	865	713, 577, 289	280
43.8	Procyanidin dimer gallate	729	577	278
44.0	Procyanidin B5	577	425, 289	280
48.5	Myricetin-3-O-glucuronide	493	317	349, 300(s), 261
50.1	Myricetin-3-O-glucoside	479	317	349, 300(s), 261
51.3	Epicatechin-3-O-gallate	441	289, 169	277
53.2	<i>trans</i> -resveratrol-3-O-glucoside	389	227	306(s), 319
55.2	Ellagic acid	301		368
57.2	Quercetin-3-O-galactoside	463	301	354, 300(s), 256
57.7	Quercetin-3-O-glucuronide	477	301	354, 300(s), 256
58.2	Astilbin	449	303	288
58.7	Quercetin-3-O-glucoside	463	301	354, 300(s), 256
60.6	Tryptophol	160		279
62.4	Kaempferol-3-O-glucoside	447	285	346, 300(s), 265
67.6	Myricetin	317		371, 300(s), 254
68.9	<i>cis</i> -resveratrol-3-O-glucoside	389	227	285
71.9	<i>trans</i> -resveratrol	227		306, 319(s)
92.7	Quercetin	301		369, 300(s), 255
95.7	<i>cis</i> -resveratrol	227		284

FIGURE 13: M^+ signals recorded by LC-Chip-QTOF of isobaric pairs of anthocyanins at m/z 611 (Cy-diglucoside (a) and Dp-*p*-coumaroylmonoglucoside (b)), at m/z 625 (Pn-diglucoside (c) and Pt-*p*-coumaroylmonoglucoside (d)), and at m/z 655 (Mv-diglucoside (e) and Mv-caffeoylmonoglucoside (f)) [49].

a C_{18} cartridge. A volume of dealcoholized wine was loaded onto the cartridge, and PAs were recovered with 10 mL of acetone/ H_2O /acetic acid 70 : 29.5 : 0.5 v/v/v solution [46].

The flow diagram in Figure 10 shows a method for fractionation of wine polyphenols by reverse-phase chromatography [43]. The more complex polyphenols recovered in the fractions 8–10 were characterized by LC/ESI-MS and MS^n negative-ion mode analysis and reported in Table 5, [57].

3. LC/MS of Grape Anthocyanins

Liquid chromatography mass spectrometry (LC/MS) coupled with multiple mass spectrometry (MS/MS and MS^n) has been widely used for structural characterization of grape anthocyanins [59] and to study the structure of new anthocyanin derivatives formed during wine aging [22, 23, 30, 36, 60–63]. LC analysis of anthocyanins is usually performed by a reverse-phase C_{18} column by performing gradient elution of compounds using a binary solvent composed of H_2O /formic acid 90 : 10 v/v and methanol/ H_2O /formic acid 50 : 40 : 10 v/v/v. The analytes are detected by recording the chromatogram at 520 nm. Due to the lack of standards

commercially available, the identification of compounds is usually performed on the basis of their column elution sequence.

A study showed that; nonacidified methanol is a solvent suitable for extraction of anthocyanins from grape skins reducing the risk of hydrolysis of acetylated compounds [64]. Alternatively, extraction by methanol/ H_2O /formic acid solution (50 : 48.5 : 1.5 v/v/v) was proposed [65]. Anthocyanins can be then purified by passing through a C_{18} cartridge: after sample loading, the nonanthocyanic phenols are eluted from the cartridge with ethyl acetate, then anthocyanins are recovered with methanol. A fast direct-injection ESI-MS/ MS analysis in positive-ion mode provides structural characterization and semiquantitative data of the anthocyanins in the extract [66]. As may be seen in the ESI-direct injection spectrum of *Clinton* grape skins extract in Figure 11, all anthocyanins show evident signal of the M^+ ion.

MS/MS and collision-induced-dissociation (CID) provide characterization of compounds. Experiments are performed by applying a supplementary radio frequency field to the endcaps of the ion trap (1–15 V) in order to make the selected ions collide with He. Precursor ions and the fragments recorded for the sample in Figure 11 are reported

FIGURE 14: MS/MS spectra of isobaric anthocyanin pairs recorded by LC-Chip/Q-TOF: Cy-diglucoside (a) and Dp-*p*-coumaroylmonoglucoside (b) with M^+ at m/z 611, Pn-diglucoside (c) and Pt-*p*-coumaroylmonoglucoside (d) with M^+ at m/z 625, and Mv-diglucoside (e) and Mv-caffeoylmonoglucoside (f) with M^+ at m/z 655 [49].

FIGURE 15: Calibration curve calculated by spiking the *Clinton* grape skins extract with Mv-3,5-O-diglucoside standard at three different concentration. Mean data of the M^+ signal area at m/z 655 extrapolated from EIC of two replicated analyses and variability bars are shown [49].

TABLE 5: Polyphenolic oligomers characterized by LC/ESI-MSⁿ in wine (fractions 8 and 9 in Figure 10) and seeds extracts.

Phenolic compounds	[M - H] ⁻	[M + H] ⁺	[M] ⁺	[M - 2H] ²⁻	(m/z)	
					MS ²	MS ³
PC2 A	577				<u>425</u> , 407, 451, 289, 559	407
PC2 B	577				<u>439</u> , 425, 393, 269, 533, 559	421, 377, 247
PC1PD1	593				<u>441</u> , 289	315, 289, 153
PC2-ethyl	605				<u>315</u> , 289, 453	229, 271, 205, 247, 191, 253, 297
PC2-G A	729				<u>407</u> , 559, 577, 451, 425, 441, 603, 289	285, 283, 297, 389, 256, 281, 243
PC2-G B	729				<u>407</u> , 559, <u>577</u> , 451, 425, 441, 603, 289	407, 451, 289, 559, 425
PC3 A	865				847, <u>577</u> , 289	451, 289
PC3 B	865				<u>695</u> , 577, 575 407, 543, 739, 713, 449, 587, 451, 407	543, 525, 677, 243, 405, 433, 451, 525, 363, 289, 501, 407, 557, 568, 391, 651
PC2-2G (A-type)	879				<u>709</u> , 727, 547, 439	557, 541, 571, 539
PC2-2G (B-type)	881				<u>729</u> , 559, 407, 711, 577	451, 407, 577, 603, 441, 559, 289
PC2PD1		883			847, 731, <u>579</u>	427, 289
PC3-ethyl A	893				<u>603</u> , 577, 451, 433, 407, 425, 315, 741	451, 433, 289, 287, 266
PC3-ethyl B	893				603, <u>577</u> , 451, 433, 407, 425, 315, 741	407, 451, 425, 541
PC3-G A	1017				<u>729</u> , 865, 847, 677, 891, 695, 577, 575, 557, 727, 739, 559, 771, 407	577, 559, 407, 425, 451, 603, 289, 467, 541
PC3-G B	1017				729, <u>865</u> , 847, 677, 891, 695, 577, 575, 557, 727, 739, 559, 771, 407	695, 407, 577, 575, 727, 425, 525, 739
PC3-G C	1017				729, 865, <u>847</u> , 677, 891, 695, 577, 575, 557, 727, 739, 559, 771, 407	677, 695, 525, 603, 451, 405
PC4 A	1153				863, <u>577</u>	451, 289
PC4 B	1153				<u>865</u> , 983, 575, 695, 739, 1027, 863, 577, 1001, 449	425, 803, 449, 451, 533, 559
PC4 C	1153				865, <u>983</u> , 575, 695, 739, 1027, 863, 577, 1001, 449	739, 577, 449, 610, 675, 447, 425, 289
PC4 D	1153				865, 983, <u>575</u> , 695, 739, 1027, 863, 577, 1001, 449	423, 449, 413, 405, 287, 289
PC6-4G or PC6PD1-2G				1168	<u>1017</u> , 729	575
PC7PD1-6G or						
PC7PD2-4G or				1616	1084, 1641	
PC7PD3-2G						
PC4	1153	1155			863, <u>577</u>	451, 289
PC3-2G A	1169				<u>1017</u> , 881, 729, 847, 999, 891, 577	729, 847, 321, 621, 495, 603, 451, 289, 577
PC3-2G B	1169				1017, <u>881</u> , 729, 847, 999, 891, 577	729, 407, 559, 711, 577, 289
PC5		1443			1425, 1291, 1155, <u>865</u> , 577	847, 577
PC2-Mv-3-glu			1069		<u>781</u>	619
Mv-3-glc-PC3	1357				<u>1067</u> , <u>779</u>	617
						601, 331
						437

PCn: n units of (epi)catechin; nG: number of galloyl units. Bold/underlined ions were subjected to next fragmentation stage [43, 57].

FIGURE 16: Fragmentation scheme proposed for two anthocyanin dimers. HCR: heterocyclic ring fission and RDA: retro-Diels-Alder fission [56].

in Table 6. A list of other monomer anthocyanins identified in extracts of other grape varieties is reported in Table 7, [66].

In general, MS^n is highly effective in differentiation also of isobaric anthocyanins. The fragment ions $[\text{M}-162]^+$, $[\text{M}-324]^+$ (formed by two consecutive losses of sugar residue), $[\text{M}-204]^+$, $[\text{M}-308]^+$, and $[\text{M}-470]^+$ (consecutive losses of *p*-coumaroylglucose and glucose) allow characterization of both monoglucoside and diglucoside compounds. Of course, the collision energy applied affects the relative abundance of diagnostic fragments. In the case of Mv-3,5-O-diglucoside and Mv-3-O-(6-caffeoyl)monoglucoside, to distinguish between two compounds by performing MS^n experiments is not possible because they have identical molecular mass and aglycone. For identification of two compounds, dissolution of the extract in deuterated water was performed to observe the different mass shifts in agreement with the different number of exchangeable acidic proton present in each molecule [66]. Direct-ESI/MS also provided semiquantitative data of anthocyanins in the extract. Quantification of compounds was performed on the calibration curves of Mv-3-O-glucoside for monoglucosides (M^+ at m/z 493) and of Mv-3,5-O-diglucoside for diglucosides (M^+ at m/z 655) being standard commercially available compounds. An Mv-3-O-glucoside 40-ppm solution in water/acetonitrile 95 : 5 v/v was used to optimize the ESI parameters in order to maximize the signals [36, 66].

Also, the capabilities of quadrupole-time-of-flight (Q-TOF) MS coupled with LC-Chip were used to distinguish between Mv-3,5-O-diglucoside and Mv-3-O-(6-O-caffeoyl)monoglucoside in *Clinton* extract, and the method

was compared with direct-infusion ESI/IT-MS [49]. LC-Chip analyses were performed using a chromatographic system composed of an enrichment column Zorbax 300 SB-C₁₈ (40 nL, 5 μm) and analytical column Zorbax 300 SB-C₁₈ (75 $\mu\text{m} \times 43 \text{ mm}$, 5 μm). Elution of compounds from the column was performed by a binary solvent mixture composed of aqueous 0.1% formic acid and methanolic 0.1% formic acid working at a flow rate of 400 nL/min. Before analysis, the grape skins extract was diluted with a loading solution (aqueous 5% methanol containing 0.1% formic acid), and 1 μL of the sample was injected. LC-Chip/ESI-QTOF MS provided the complete anthocyanin fingerprint of the sample in less than 5 minutes with practically no solvent consumption. Neither MS/MS was necessary for identification of isobaric compounds, nor deuterium exchange experiments to distinguish between compounds having the same aglycone. The fast separation bypassed also the problem of Pt-3-O-(6-O-acetyl)monoglucoside and Dp-3,5-O-diglucoside quantification of direct-infusion ESI/IT MS due to overlapping of their signals with matrix interferences. The high specificity of LC-Chip/Q-TOF-MS was due to highly reproducible retention times (Chip-chromatography reduces the problems arising from dead volumes, eluent flow constancy, and ESI condition stabilities), highly effective and reproducible collisional experiments, accurate mass measurements, and consequent elemental formula determination of the precursor and fragment ions. Total ion chromatogram (TIC) and extracted ion chromatogram (EIC) of the three isobaric compound pairs at m/z 611, 625, and 655 identified

TABLE 6: Fragments from M^+ ions of anthocyanins in *Clinton* grape skin extract produced by direct-ESI and MSⁿ [66].

Anthocyanin	<i>m/z</i>			
	M ⁺	[M – C ₆ H ₁₀ O ₅] ⁺		
Malvidin-3-O-monoglucoside	493	331		
Petunidin-3-O-monoglucoside	479	317		
Delphinidin-3-O-monoglucoside	465	303		
Peonidin-3-O-monoglucoside	463	301		
Cyanidin-3-O-monoglucoside	449	287		
	M ⁺	[M – C ₈ H ₁₂ O ₆] ⁺		
Malvidin-3-O-(6-O-acetyl)monoglucoside	535	331		
Petunidin-3-O-(6-O-acetyl)monoglucoside	521	317		
Delphinidin-3-O-(6-O-acetyl)monoglucoside	507	303		
Peonidin-3-O-(6-O-acetyl)monoglucoside	505	301		
Cyanidin-3-O-6-O-(acetyl)monoglucoside	491	287		
	M ⁺	[M – C ₁₅ H ₁₆ O ₇] ⁺		
Malvidin-3-O-(6-O-p-coumaroyl)monoglucoside	639	331		
Petunidin-3-O-(6-O-p-coumaroyl)monoglucoside	625	317		
Delphinidin-3-O-(6-O-p-coumaroyl)monoglucoside	611	303		
Peonidin-3-O-(6-O-p-coumaroyl)monoglucoside	609	301		
Cyanidin-3-O-(6-O-p-coumaroyl)monoglucoside	595	287		
	M ⁺	[M – C ₆ H ₁₀ O ₅] ⁺	[M – 2(C ₆ H ₁₀ O ₅)] ⁺	
Malvidin-3,5-O-diglucoside	655	493	331	
Petunidin-3,5-O-diglucoside	641	479	317	
Delphinidin-3,5-O-diglucoside	627	465	303	
Peonidin-3,5-O-diglucoside	625	463	301	
Cyanidin-3,5-O-diglucoside	611	449	287	
	M ⁺	[M – C ₆ H ₁₀ O ₅] ⁺	[M – C ₁₅ H ₁₆ O ₇] ⁺	[M – C ₁₅ H ₁₆ O ₇ –C ₆ H ₁₀ O ₅] ⁺
Malvidin-3-(6-O-p-coumaroyl),5-O-diglucoside	801	639	493	331
Petunidin-3-(6-O-p-coumaroyl),5-O-diglucoside	787	625	479	317
Delphinidin-3-(6-O-p-coumaroyl),5-O-diglucoside	773	611	465	303
Cyanidin-3-(6-O-p-coumaroyl),5-O-diglucoside	757	595	449	287
	M ⁺	[M – C ₁₅ H ₁₆ O ₈] ⁺		
Malvidin-3-O-(6-O-caffeoyl)monoglucoside	655	331		

TABLE 7: Monomer anthocyanins identified by LC/ESI-MS/MS analysis in skin extract and juice of different grape cultivars [24, 69].

Anthocyanin	m/z $[M]^+$	Cultivar
Cy-3-O-pentoside	419	Casavecchia
Pg-3-O-glucoside	433	Concord, Salvador, Rubired
Cy-3-O-(6-O-acetyl)pentoside	461	Casavecchia
Cy-3-O-(6-O-p-coumaryl)pentoside	565	Casavecchia
Dp-3-O-glucoside-pyruvic acid	533	Isabelle
Dp-3-O-(6-O-p-coumaryl)glucoside-pyruvic acid	679	Isabelle
Pn-3-O-glucoside-acetaldehyde	487	Isabelle, Pallagrello
Mv-3-O-glucoside-acetaldehyde	517	Isabelle
Pt-3-O-(6-O-p-caffeoyl)-5-O-diglucoside	803	Isabelle, Casavecchia
Dp-3-O-(6-O-acetyl)-5-O-diglucoside	669	Isabelle
Dp-3-O-(6-O-feruloyl)-5-O-diglucoside	803	Isabelle, Casavecchia
Pn-3-O-(6-O-p-coumaryl)-5-O-diglucoside	771	Concord, Salvador, Isabelle, Casavecchia

Pg: pelargonidin; Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin.

FIGURE 17: UPLC/MS total ion chromatogram (TIC) (a) and precursor-ion chromatograms from anthocyanin analysis of *Bacò* 30-12 grape skins extract. (b) Precursor-ion chromatogram of fragment at m/z 331 (corresponding to Mv); (c) precursor-ion chromatogram of fragment at m/z 317 (Pt); (d) precursor-ion chromatogram of fragment at m/z 303 (Dp); (e) precursor-ion chromatogram of fragment at m/z 301 (Pn); (f) precursor-ion chromatogram of fragment at m/z 287 (Cy) [58].

in the analysis of *Clinton* grape skins extract are showed in Figure 12.

Reverse-phase LC-Chip allowed the separation of all the isobaric pairs of compounds with an elution sequence from the column linked to the polarity of compounds: first elute the more polar diglucosides, followed by monoglucosides, and finally the less polar acylated monoglucosides (acetates and *p*-coumarates, resp.). The anthocyanins identified in the *Clinton* extract by LC-Chip/Q-TOF and direct-ESI/IT-MS analysis are reported in Table 8.

To distinguish between isobaric pairs of anthocyanins, also accurate mass measurements were performed. Figure 13 shows the Q-TOF signals recorded after LC-Chip separation of isobaric pairs at nominal mass m/z 611 (Cy-diglucoside

and Dp-*p*-coumaroylmonoglucoside), m/z 625 (Pn-diglucoside and Pt-*p*-coumaroylmonoglucoside), and m/z 655 (Mv-diglucoside and Mv-caffeoylmonoglucoside). For these ions, the Q-TOF system used did not provide sufficient resolution to distinguish between Cy-3,5-O-diglucoside ($C_{27}H_{31}O_{16}$, MW 611.1612) and Dp-3-O-(6-O-*p*-coumaroyl)monoglucoside ($C_{30}H_{27}O_{14}$, MW 611.1401), Pn-3,5-O-diglucoside ($C_{28}H_{33}O_{16}$, MW 625.1769) and Pt-3-O-(6-O-*p*-coumaroyl)monoglucoside ($C_{31}H_{29}O_{14}$, MW 625.1557), or Mv-3,5-O-diglucoside ($C_{29}H_{35}O_{17}$, MW 655.1874) and Mv-3-O-(6-O-caffeoyl)monoglucoside ($C_{32}H_{31}O_{15}$, MW 655.1663) because a resolution at least 30,000 is necessary. MS/MS allowed to confirm identification of the compounds with M^+ at m/z 611 and m/z 625 (on the basis of their

FIGURE 18: MS/MS fragmentation scheme proposed for (epi)catechin-Mv-3-glu B-type linkage (M^+ at m/z 781) and for Mv-3-glu-(epi)catechin A-type linkage (M^+ at m/z 783) [67, 68]. RDA: retro-Diels-Alder fission.

FIGURE 19: Possible structures proposed for the M^+ species at m/z 1273: (a) (E)C-MvG-Mv+G trimer in flavene-flavylium form (B-type linkage) and (b) (E)C-MvG-Mv+G trimer in flavan-flavylium form (A-type linkage) [60].

FIGURE 20: Mass spectra of a *Cabernet Sauvignon* wine recorded by application of spray capillary voltage 3 kV (a) and 0.5 kV (b). *: main anthocyanin signals [70].

different aglycone ions), but only chip separation allowed differentiation of two isobaric compounds with the same aglycone moiety (nominal mass 655 Da).

Figure 14 shows the MS/MS mass spectra of the isobaric anthocyanin pairs Cy-diglucoside and Dp-*p*-coumaroylmonoglucoside (M^+ at m/z 611), Pn-diglucoside and Pt-*p*-coumaroylmonoglucoside (M^+ at m/z 625), and Mv-diglucoside and Mv-caffeoylmonoglucoside (M^+ at m/z 655). The spectra (a), (c), and (e) are characteristic of diglucoside compounds and show the ions formed by 162 Da loss corresponding to glucose residue at m/z 449, 463, and 493 for Cy-diglucoside, Pn-diglucoside, and Mv-diglucoside, respectively, and the aglycone ions at m/z 287, 301, and m/z

331. The spectra (b), (d), and (f), characteristic of cinnamoylmonoglucoside derivatives, show the aglycones of Dp-*p*-coumaroylmonoglucoside, Pt-*p*-coumaroylmonoglucoside, and Mv-caffeoylmonoglucoside at m/z 303, 317, and m/z 331, respectively.

Concentration of anthocyanins in the extract was determined on the calibration curve calculated by spiking the sample with Mv-3,5-O-diglucoside standard at three different concentrations. The resulting calibration curve is showed in Figure 15.

Quantitative percentage data of LC-Chip/ESI-QTOF-MS and ESI/IT-MS analysis of *Clinton* extract are reported in Table 8. The two methods showed good agreement, except

FIGURE 21: Direct-infusion pneumatic spray (DIPS with capillary voltage 0.5 kV) of a *Cabernet Sauvignon* wine aged several years. Mv: malvidin; IS: internal standard Mv-3,5-diglucoside [70].

FIGURE 22: Signals of PAs in the MALDI-TOF spectrum of a *Cabernet Sauvignon* grape seeds extract.

TABLE 8: Anthocyanins identified in *Clinton* grape skins extract by direct-ESI/IT-MS and LC-Chip/ESI-QTOF-MS analysis performed in positive-ion mode (LC-Chip retention times are reported). Data are expressed as relative percentage of M^+ signal of compound with respect to M^+ signal of Mv-3-O-monoglucoside at m/z 611 and m/z 493.

Compound	MW	Direct-ESI/IT-MS	RT (min)	LC-Chip/ESI-Q-TOF-MS
		M^+ height % Relative to Mv monoglucoside		M^+ area % Relative to Mv monoglucoside
Cyanidin-3-O-monoglucoside	449	9	3.794	11
Peonidin-3-O-monoglucoside	463	33	3.869	52
Delphinidin-3-O-monoglucoside	465	35	3.744	38
Petudinin-3-O-monoglucoside	479	44	3.794	39
Cyanidin-3-O-(6-O-acetyl)monoglucoside	491	2	4.095	tr
Malvidin-3-O-monoglucoside	493	100	3.869	100
Peonidin-3-O-(6-O-acetyl)monoglucoside	505	10	4.070	6
Delphinidin-3-O-(6-O-acetyl)monoglucoside	507	6	3.944	2
Petudinin-3-O-(6-O-acetyl)monoglucoside	521	<u>23</u>	3.995	5
Malvidin-3-O-(6-O-acetyl)monoglucoside	535	36	4.045	30
Cyanidin-3-(6-O- <i>p</i> -coumaryl)monoglucoside	595	3	4.220	5
Peonidin-3-(6-O- <i>p</i> -coumaryl)monoglucoside	609	6	4.295	18
Cyanidin-3,5-O-diglucoside	611	19 (m/z 611)	3.644	3
Delphinidin-3-(6-O- <i>p</i> -coumaryl)monoglucoside	611		4.170	15
Peonidin-3,5-O-diglucoside	625	14 (m/z 625)	3.719	3
Petudinin-3-(6-O- <i>p</i> -coumaryl)monoglucoside	625		4.220	3
Delphinidin-3,5-O-diglucoside	627	<u>35</u>	3.594	2
Malvidin-3-(6-O- <i>p</i> -coumaryl)monoglucoside	639	39	4.270	48
Petudinin-3,5-O-diglucoside	641	16	3.669	14
Malvidin-3,5-O-diglucoside	655	24 (m/z 655)	3.719	26
Malvidin-3-(6-O-cafeoyl)monoglucoside	655		4.170	1
Cyanidin-3-(6-O- <i>p</i> -coumaryl),5-O-diglucoside	757	2	3.995	1
Delphinidin-3-(6-O- <i>p</i> -coumaryl),5-O-diglucoside	773	2	3.944	2
Petudinin-3-(6-O- <i>p</i> -coumaryl),5-O-diglucoside	787	2	3.995	3
Malvidin-3-(6-O- <i>p</i> -coumaryl),5-O-diglucoside	801	2	4.045	3

tr: trace [49].

for Pt-3-O-(6-O-acetyl)monoglucoside (m/z 521) and Dp-3,5-O-diglucoside (m/z 627) for which the M^+ signal area resulted overestimated in the ESI/IT-MS analysis. CID of the species at m/z 627 in the ESI/MS spectrum, other than the ions at m/z 465 and 303 corresponding to Dp-3,5-O-diglucoside, produced the ion at m/z 317 as the most intense signal probably corresponding to petunidin or protonated isorhamnetin revealing the overlapping of at least two compounds. CID spectrum of the ion at m/z 521, other than the aglycone base peak signal at m/z 317, showed two intense signals at m/z 522 and 488 with relative abundance of about 40% each. MS^3 of the ion at m/z 488 showed that at least two isobaric compounds were present in the extract.

TOF/MS was not suitable to distinguish between isobaric pairs of compounds such as protonated isorhamnetin glucoside and petunidin glucoside ($C_{22}H_{23}O_{12}$, exact mass 479.1190), and protonated kaempferol monoglucoside and cyanidin monoglucoside ($C_{21}H_{21}O_{11}$, exact mass 449.1084). Intensities of the signals at m/z 449 and m/z 479 recorded by direct-ESI/IT-MS and LC-Chip/ESI-QTOF-MS are similar (Table 8), inferring that probably protonated flavonols

present in the extract were not separated from the chromatographic chip used.

In grape skins, also the oligomeric anthocyanins listed in Table 9 were identified [56, 59]. Figure 16 shows the fragmentation scheme proposed for two anthocyanin dimers.

In a recent work anthocyanins of 21 hybrid red grape varieties produced by crossing of different *Vitis vinifera*, *riparia*, *labrusca*, *Lincecumii*, and *rupestris* species were studied [58]. In general, hybrid grapes are characterized by peculiar contents of anthocyanins, often qualitatively and quantitatively different—and superior—to the *V. vinifera* varieties [66, 71–77]. Also due to the increasing industrial demand for natural colorants, the knowledge of their composition may be useful for industrial purposes [78, 79].

The study was performed by using an ultraperformance liquid chromatography and triple quadrupole mass spectrometry system (UPLC/MS). Precursor-ion analysis of the anthocyanidin and monoglucoside-anthocyanin fragments produced by CID was performed. Twenty-four compounds were identified using two different experimental conditions: precursor-ions scan of the aglycone fragments produced at

TABLE 9: Oligomeric anthocyanins identified in *Shiraz* grape skins.

m/z	Assignment
617 ^F	MvCy
631 ^F	MvPn
633 ^F	MvDp
647 ^F	MvPt
661 ^F	MvMv
779 ^F	MvCy + G
793 ^F	MvPn + G
795 ^F	MvDp + G
809 ^F	MvPt + G
823 ^F	MvMv + G
941 ^M	MvCy + 2G
955 ^M	MvPn + 2G
957 ^M	MvDp + 2G
971 ^M	MvPt + 2G
985 ^M	MvMv + 2G
1087 ^M	MvCy + G-pCG
1101 ^M	MvPn + G-pCG
1103 ^M	MvDp + G-pCG
1117 ^M	MvPt + G-pCG
1131 ^M	MvMv + G-pCG
1271 ^F	MvMvCy + 2G
1285 ^F	MvMvPn + 2G
1287 ^F	MvMvDp + 2G
1301 ^F	MvMvPt + 2G
1315 ^F	MvMvMv + 2G
1417 ^F	MvMvCy + G-pCG
1431 ^F	MvMvPn + G-pCG
1433 ^F	MvMvDp + G-pCG
1433 ^M	MvMvCy + 3G
1447 ^F	MvMvPt + G-pCG
1447 ^M	MvMvPn + 3G
1449 ^M	MvMvDp + 3G
1461 ^F	MvMvMv + G-pCG
1463 ^M	MvMvPt + 3G
1477 ^M	MvMvMv + 3G
1579 ^M	MvMvCy + 2G-pCG
1593 ^M	MvMvPn + 2G-pCG
1595 ^M	MvMvDp + 2G-pCG
1609 ^M	MvMvPt + 2G-pCG
1623 ^M	MvMvMv + 2G-pCG

F: fragment ion; M: molecular ion. Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; G: glucose, pCG: *p*-coumaroylglucoside [56].

a collision energy of 4 eV and precursors scan of monoglucoside anthocyanin fragments produced at collision energy 25 eV. Analysis of precursor ions of these fragments was possible because usually no fragmentation occurs in the

glucose and acyl group linkage, nor formation of acylglucoside anthocyanins was observed in neutral-loss analysis [80]. The samples were subdivided into two groups on the basis of their anthocyanin profiles which were characterized by the substantial presence or scarce presence of diglucoside compounds, respectively. Analysis of precursor ions showed to be a highly selective method: by monitoring each aglycone, the signals of all corresponding derivatives are detected. This approach can be used for selective study of particular anthocyanidin derivatives in the sample; for example, by monitoring the product ion at m/z 331 (corresponding to Mv), the signals of precursors at m/z 493 (the monoglucoside derivative), m/z 535 (acetyl monoglucoside), m/z 639 (*p*-coumaroylmonoglucoside), m/z 655 (diglucoside), and m/z 801 (*p*-coumaroyl diglucoside) were detected. In addition, precursor-ion analysis enhances the signal-to-noise ratio, allowing more sensitivity in analysis of anthocyanins in complex matrices [80].

Figure 17 shows the TIC and precursor-ion chromatograms of aglycone fragments from analysis of a hybrid grape sample (*Bacò 30-12*). Three precursor ions for Dp, Pn, and, Cy anthocyanidin fragments and five precursor ions for Mv and Pt were found, leading to identification of the 20 anthocyanins (13 monoglucosides and 7 diglucosides) reported in Table 10.

The precursors identified by precursor-ion analysis of monoglucoside anthocyanins are reported in Table 11. Identification of diglucoside compounds found in precursor-ion analysis of aglycone fragments was confirmed, and the signals of four additional diglucoside compounds were also found [Mv-3-O-(6-O-acetyl)diglucoside at m/z 697, Dp-3,5-diglucoside at m/z 627, Pn-3-O-(6-O-*p*-coumaroyl)diglucoside at m/z 771, and Cy-3-O-(6-O-*p*-coumaroyl)diglucoside at m/z 757], leading to identification of total 11 diglucoside anthocyanins. Therefore, the coupling of UPLC and precursor-ion analysis with monitoring of monoglucosides resulted a highly specific method for selective detection of diglucoside compounds.

4. LC/MS of Anthocyanin Derivatives in Wine

LC/MS analysis of anthocyanin derivatives in wine can be performed by direct injection of the sample without prior sample preparation, and several methods with different chromatographic conditions were proposed by this approach. Table 12 shows the compounds identified in *Graciano*, *Tempranillo*, *Cabernet Sauvignon*, and *Primitivo* wines. Alternatively, a previous sample purification can be performed on a C_{18} cartridge recovering anthocyanins with methanol [81].

Several methods for isolation and fractionation of oligomeric pigments in the analysis of pyranoanthocyanins and anthocyanin derivatives in wine were proposed [22, 33]. Anthocyanin-flavanol derivatives can be characterized by MS/MS experiments (Table 12). For example, Figure 18 shows the fragmentation schemes proposed for (epi)catechin-Mv-3-glu (M^+ at m/z 781) and for Mv-3-glu-(epi)catechin with A-type linkage (M^+ at m/z 783).

A list of anthocyanin derivatives identified in wines at different aging stages is reported in Table 13. As may be seen,

TABLE 10: Precursors of the aglycone fragment ions identified by UPLC/MS precursor-ion analysis in the study of 21 hybrid grape varieties.

Varieties	Precursor ions of <i>m/z</i> 331 (Mv)			Precursor ions of <i>m/z</i> 317 (Pt)			Precursor ions of <i>m/z</i> 303 (Dp)			Precursor ions of <i>m/z</i> 301 (Pn)			Precursor ions of <i>m/z</i> 287 (Cy)		
	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound
Bacò 1	655	1.65	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	493	2.06	Monoglu.	625	2.79	(<i>p</i> -coum.)-Monoglu.									
	801	2.60	(<i>p</i> -coum.)-diglu.												
	639	2.96	(<i>p</i> -coum.)-monoglu.												
Bacò 30-12	655	1.65	Diglu.	641	1.41	Diglu.	465	1.57	Monoglu.	625	1.63	Diglu.	611	1.43	Diglu.
	493	2.06	Monoglu.	479	1.82	Monoglu.	773	2.24	(<i>p</i> -coum.)-diglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	801	2.63	(<i>p</i> -coum.)-diglu.	625	2.79	(<i>p</i> -coum.)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	609	2.58	(<i>p</i> -coum.)-monoglu.	595	2.74	(<i>p</i> -coum.)-monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.												
Bertille Seivè 1808	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.				449	1.79	Monoglu.
							611	2.62	(<i>p</i> -coum.)-monoglu.						
Bertille Seivè 4825	655	1.65	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	611	1.43	Diglu.
	493	2.06	Monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
Clinton	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.				611	2.62	(<i>p</i> -coum.)-monoglu.	609	2.58	(<i>p</i> -coum.)-monoglu.	595	2.74	(<i>p</i> -coum.)-monoglu.
										463	2.04	Monoglu.	449	1.79	Monoglu.
Couderc 25	655	1.61	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	611	1.43	Diglu.
	493	2.06	Monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.										595	2.74	(<i>p</i> -coum.)-monoglu.
													449	1.79	Monoglu.
Galibert 238-35	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	535	2.56	(Acetyl)-monoglu.	521	2.35	(Acetyl)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	625	1.63	Diglu.	611	1.43	Diglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.				463	2.04	Monoglu.	449	1.79	Monoglu.
													595	2.74	(<i>p</i> -coum.)-monoglu.
Seibel 10878	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.	521	2.36	(Acetyl)-monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.						
Seibel 8357	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	463	2.04	Monoglu.	449	1.79	Monoglu.
	801	2.60	(<i>p</i> -coum.)-diglu.	521	2.30	(Acetyl)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	625	1.63	Diglu.	449	1.79	Monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.	787	2.45	(<i>p</i> -coum.)-diglu.				505	2.54	(Acetyl)-monoglu.			
				625	2.79	(<i>p</i> -coum.)-monoglu.									
Seibel 8745	655	1.65	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	449	1.79	Monoglu.
	493	2.06	Monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.						
Seyve Villard 12-347	655	1.65	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	449	1.79	Monoglu.
	493	2.06	Monoglu.				611	2.62	(<i>p</i> -coum.)-monoglu.						
	639	2.96	(<i>p</i> -coum.)-monoglu.												
Seyve Villard 12-390	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	449	1.79	Monoglu.
	535	2.56	(Acetyl)-monoglu.	521	2.35	(Acetyl)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	463	2.04	Monoglu.	595	2.74	(<i>p</i> -coum.)-monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.									
				479	1.82	Monoglu.	465	2.62	Monoglu.						
Seyve Villard 23-369	493	2.06	Monoglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	449	1.79	Monoglu.
	535	2.56	(Acetyl)-monoglu.	521	2.35	(Acetyl)-monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	463	2.04	Monoglu.	595	2.74	(<i>p</i> -coum.)-monoglu.
	639	2.96	(<i>p</i> -coum.)-monoglu.	625	2.79	(<i>p</i> -coum.)-monoglu.									
				479	1.82	Monoglu.	465	2.62	Monoglu.						
Seyve Villard 23-399	493	2.06	Diglu.	641	1.41	Diglu.	465	1.57	Monoglu.	625	1.63	Diglu.	611	1.40	Diglu.
	655	1.65	Diglu.	479	1.82	Monoglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	449	1.79	Monoglu.	449	1.79	Monoglu.
	493	2.06	Monoglu.												
				625	2.79	(<i>p</i> -coum.)-monoglu.							595	2.74	(<i>p</i> -coum.)-monoglu.
Seyve Villard 23-512	655	1.65	Diglu.	479	1.82	Monoglu.	465	1.57	Monoglu.	625	1.63	Diglu.	611	1.40	Diglu.
	493	2.06	Monoglu.	641	1.41	Diglu.	611	2.62	(<i>p</i> -coum.)-monoglu.	449	1.79	Monoglu.	449	1.79	Monoglu.
	655	1.65	Diglu.	479	1.82	Monoglu.									
	801	2.60	(<i>n</i> -coum.)-diglu.	625	2.79	(<i>p</i> -coum.)-monoglu.							595	2.74	(<i>p</i> -coum.)-monoglu.

TABLE 10: Continued.

Varieties	Precursor ions of m/z 331 (Mv)			Precursor ions of m/z 317 (Pt)			Precursor ions of m/z 303 (Dp)			Precursor ions of m/z 287 (Cy)		
	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound
Seyve Villard 29-522	655	1.65	Diglucl.	479	1.82	Monogluc.	465	1.57	Monogluc.			
	493	2.06	Monogluc.	625	2.79	(<i>p</i> -coum.)-monogluc.	611	2.62	(<i>p</i> -coum.)-monogluc.			
	801	2.60	(<i>p</i> -coum.)-diglucl.									
	639	2.96	(<i>p</i> -coum.)-monogluc.									
Terzi 100-31	655	1.65	Diglucl.	479	1.82	Monogluc.	465	1.57	Monogluc.	625	1.63	Diglucl.
	493	2.06	Monogluc.							463	2.04	Monogluc.
	639	2.96	(<i>p</i> -coum.)-monogluc.									
Terzi 108-6	493	2.06	Monogluc.	479	1.82	Monogluc.	465	1.57	Monogluc.	625	1.63	Diglucl.
	639	2.96	(<i>p</i> -coum.)-monogluc.				611	2.62	(<i>p</i> -coum.)-monogluc.	463	2.04	Monogluc.
	655	1.65	Diglucl.	479	1.82	Monogluc.	465	1.57	Monogluc.	463	2.04	Monogluc.
Terzi 97-46	493	2.06	Monogluc.	787	2.45	(<i>p</i> -coum.)-diglucl.	611	2.62	(<i>p</i> -coum.)-monogluc.			
	535	2.60	(Acetyl)-monogluc.	625	2.79	(<i>p</i> -coum.)-monogluc.						
	639	2.96	(<i>p</i> -coum.)-monogluc.									
	493	2.06	Monogluc.	479	1.82	Monogluc.	465	1.57	Monogluc.	463	2.04	Monogluc.
Unknown	639	2.96	(<i>p</i> -coum.)-monogluc.	625	2.79	(<i>p</i> -coum.)-monogluc.	611	2.62	(<i>p</i> -coum.)-monogluc.	609	2.95	(<i>p</i> -coum.)-monogluc.
										449	1.79	Monogluc.

Rt: column retention time (min) [58].

TABLE 11: Precursors of the monoglucoside anthocyanin fragment ions identified by UPLC/MS precursor-ion analysis in 21 hybrid grape varieties.

Varieties	Precursor ions of <i>m/z</i> 493 (Mv)			Precursor ions of <i>m/z</i> 479 (Pt)			Precursor ions of <i>m/z</i> 465 (Dp)			Precursor ions of <i>m/z</i> 463 (Pn)			Precursor ions of <i>m/z</i> 449 (Cy)		
	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound	M ⁺	Rt	Compound
Bacò 1	655	1.65	Diglu.	641	1.40	Diglu.	773	2.32	(<i>p</i> -coum.)-digluc.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.				770	2.65	(<i>p</i> -coum.)-digluc.			
Bacò 30-12	655	1.65	Diglu.	641	1.40	Diglu.	773	2.32	(<i>p</i> -coum.)-digluc.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.												
Bertille Seivè 1808	655	1.65	Diglu.	641	1.40	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
Bertille Seivè 4825	655	1.65	Diglu.	641	1.40	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.							770	2.65	(<i>p</i> -coum.)-digluc.	757	2.41	(<i>p</i> -coum.)-digluc.
Burdin 4077	655	1.65	Diglu.	641	1.47	Diglu.									
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.									
	655	1.65	Diglu.	641	1.47	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
Clinton	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.									
Couderc 25	655	1.65	Diglu.	641	1.40	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.60	(<i>p</i> -coum.)-digluc.												
Galibert	655	1.65	Diglu.												
238-35	801	2.61	(<i>p</i> -coum.)-digluc.												
Seibel 10878															
Seibel 8357	655	1.65	Diglu.	641	1.47	Diglu.	773	2.32	(<i>p</i> -coum.)-digluc.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.				770	2.65	(<i>p</i> -coum.)-digluc.	757	2.45	(<i>p</i> -coum.)-digluc.
Seibel 8745	655	1.65	Diglu.	641	1.40	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.				773	2.32	(<i>p</i> -coum.)-digluc.	770	2.65	(<i>p</i> -coum.)-digluc.			
Seyve Villard 12.347	655	1.65	Diglu.	641	1.47	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.									
Seyve Villard 12.390	655	1.65	Diglu.	641	1.40	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.							770	2.65	(<i>p</i> -coum.)-digluc.			
Seyve Villard 23.369	655	1.65	Diglu.	641	1.40	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
Seyve Villard 23.399	655	1.65	Diglu.	641	1.47	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.									
Seyve Villard 23.512	655	1.65	Diglu.	641	1.47	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
	697	2.06	(Acetyl)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.				770	2.65	(<i>p</i> -coum.)-digluc.			
Seyve Villard 29.522	801	2.61	(<i>p</i> -coum.)-digluc.												
	655	1.65	Diglu.	641	1.47	Diglu.	627	1.27	Diglu.	625	1.65	Diglu.	611	1.44	Diglu.
Terzi 100.31	801	2.61	(<i>p</i> -coum.)-digluc.												
	655	1.65	Diglu.	641	1.40	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
Terzi 108.6	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.	627	1.27	Diglu.	770	2.65	(<i>p</i> -coum.)-digluc.	611	1.44	Diglu.
							773	2.32	(<i>p</i> -coum.)-digluc.	625	1.65	Diglu.	757	2.41	(<i>p</i> -coum.)-digluc.
Terzi 97.46	655	1.65	Diglu.	641	1.47	Diglu.	627	1.27	Diglu.	625	1.66	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.	773	2.32	(<i>p</i> -coum.)-digluc.						
Unknown	655	1.65	Diglu.	641	1.47	Diglu.				625	1.65	Diglu.	611	1.44	Diglu.
	801	2.61	(<i>p</i> -coum.)-digluc.	787	2.45	(<i>p</i> -coum.)-digluc.				770	2.65	(<i>p</i> -coum.)-digluc.			

Rt: column retention time (min) [58].

TABLE 12: Anthocyanin derivatives identified by positive-ion LC/ESI-MS analysis in *Graciano*, *Tempranillo*, *Cabernet Sauvignon*, and *Primitivo* wines [36, 82].

Compound	[M] ⁺	m/z	
		Main MS ² fragments	Main MS ³ fragments
(epi)catechin-peonidin-3-O-glucoside	751	589	571, 437, 463
(epi)catechin-malvidin-3-O-glucoside	781	619	601, 493, 467
Delphinidin-3-O-glucoside	465	303	/
Di(epi)catechinMalvidin-3-O-glucoside	1069	907, 781, 619	/
Cyanidin-3-O-glucoside	449	287	/
(epi)catechin-malvidin-3-O-glucoside	781	619	601, 493, 467
Di(epi)catechin-malvidin-3-O-glucoside	1069	907, 781, 619	/
Petunidin-3-O-glucoside	479	317	/
Peonidin-3-O-glucoside	463	301	/
Malvidin-3-O-glucoside	493	331	/
Malvidin-3-O-glucoside pyruvate	561	399	/
Malvidin-3-O-glucoside acetaldehyde	517	355	/
Malvidin-3-O-glucoside-8-ethyl-(epi)catechin	809	647, 519, 357	/
Malvidin-3-O-glucoside-8-ethyl-(epi)catechin	809	647, 519, 357	/
Malvidin-3-O-glucoside-4-vinyl-di(epi)catechin	1093	931, 803	641
Malvidin-3-O-glucoside-8-ethyl-(epi)catechin	809	647, 519, 357	/
Malvidin-3-O-glucoside-4-vinyl-di(epi)catechin	1093	931, 803	641
Malvidin-3-O-glucoside-8-ethyl-(epi)catechin	809	647, 519, 357	/
Malvidin-3-(6-O-p-coumaroylglucoside)-(epi)catechin	927	619	601, 493, 467
Malvidin-3-(6-O-p-coumaroylglucoside) pyruvate	707	399	/
Peonidin-3-(6-O-acetylglucoside)	505	301	/
Malvidin-3-(6-O-acetylglucoside)	535	331	/
Delphinidin-3-(6-O-p-coumaroylglucoside)	611	303	/
Malvidin-3-(6-O-acetylglucoside)-4-vinyl-(epi)catechin	847	643	491
Malvidin-3-(6-O-caffeoylglucoside)	655	331	/
Cyanidin-3-(6-O-p-coumaroylglucoside)	595	287	/
Petunidin-3-(6-O-p-coumaroylglucoside)	625	317	/
Malvidin-3-(6-O-p-coumaroylglucoside)	639	331	/
Malvidin-3-(6-O-p-coumaroylglucoside)-4-vinyl-di(epi)catechin	1239	931, 641	641
Malvidin-3-O-glucoside-4-vinyl-(epi)catechin	805	643	491
Peonidin-3-(6-O-p-coumaroylglucoside)-8-ethyl-(epi)catechin	925	635, 617, 327	/
Malvidin-3-(6-O-p-coumaroylglucoside)-8-ethyl-(epi)catechin	955	665, 357	/
Peonidin-3-(6-O-p-coumaroylglucoside)	609	301	/
Malvidin-3-(6-O-p-coumaroylglucoside)	639	331	/
Malvidin-3-O-glucoside-4-vinylcatechol	625	463	/
Malvidin-3-O-glucoside-4-vinyl-(epi)catechin	805	643	491
Malvidin-3-(6-O-p-coumaroylglucoside)-4-vinyl-(epi)catechin	951	643	491
Malvidin-3-(6-O-p-coumaroylglucoside)-4-vinyl-(epi)catechin	951	643	491
Malvidin-3-O-glucoside-4-vinylphenol	609	447	/
Malvidin-3-O-glucoside-4-vinylguaiacol	639	477	/
Malvidin-3-(6-O-p-coumaroylglucoside)-8-ethyl-(epi)catechin	955	665, 357	/
Malvidin-3-O-glucoside-(epi)catechin	781	619	/
Delphinidin-3-O-glucoside	465	303	/
Peonidin-3-O-glucoside pyruvate	531	369	/
Malvidin-3-O-glucoside pyruvate	561	399	/
Malvidin-3-(6-O-acetylglucoside) pyruvate	603	399	/
Malvidin-3-O-glucoside-8-ethyl-(epi)catechin	809	647, 519, 357	/
Malvidin-3-(6-O-p-coumaroylglucoside) pyruvate	707	399	/
Peonidin-3-(6-O-caffeoylglucoside)	625	301	/

TABLE 12: Continued.

Compound	m/z		
	$[M]^+$	Main MS ² fragments	Main MS ³ fragments
Malvidin-3-(6-O-caffeoylglucoside)	655	331	
Malvidin-3-O-glucoside-4-vinyl-catechin	805	643	
Malvidin-3-(6-O-p-coumaroylglucoside)-8-ethyl-(epi)catechin	955	665, 357	
Malvidin-3-(6-O-acetylglucoside)-4-vinyl-catechin	847	643	
Malvidin-3-O-glucoside-4-vinylcatechol	625	463	
Malvidin-3-(6-O-acetylglucoside)-4-vinyl-epicatechin	847	643	
Malvidin-3-O-glucoside-4-vinyl-epicatechin	805	643	
Malvidin-3-O-glucoside-4-vinylphenol	609	447	
Malvidin-3-O-glucoside-4-vinylguaiacol	639	477	
Malvidin-3-(6-O-acetylglucoside)-4-vinylphenol	651	447	
Malvidin-3-(6-O-p-coumaroylglucoside)-4-vinylphenol	755	447	

ethyl-bridge derivatives, pyranoanthocyanins, and pigments formed by anthocyanin-flavanol linkage are included. Some of these compounds are already present in wine in the first aging stage and disappear in the time, and others are formed with long time aging.

Also, oligomeric pigments F-A-A⁺ type (F, flavanol; A, anthocyanin) were identified in wines and characterized by ESI/MSⁿ. Table 14 reports the compounds identified in *Tempranillo* aged wines. Possible structures proposed for the M⁺ species at m/z 1273 are shown in Figure 19, [60].

A fast and selective method for screening of the anthocyanic composition of wine was recently proposed [70]. Analysis of wine extract was performed by direct-infusion ESI-MS/MS operating in positive-ion mode with application of a low spray capillary voltage (0.5 kV). The high selectivity of the method towards anthocyanins is due to the following: by operating far from ESI conditions, any process related to electrospray ionization occurs (these processes taking place with capillary voltages up 3 kV [83]), and only the species already present in ionic form in the sprayed solution can be detected [84]. In ESI conditions, simultaneous detection of anthocyanins, their derivatives, and protonated ions of other wine components can lead to a quite complex panorama, with possible occurrence of matrix effects, favouring the detection of nonanthocyanic compounds. Operating with a low spraying capillary voltage, the protonation of these molecules is strongly reduced, or avoided, so allowing to obtain directly a fingerprint of the cations already present in the sample. As a matter of fact, by decreasing the spray capillary voltage, a dramatic increase of selectivity toward anthocyanins was observed and their peaks become the most abundant (Figure 20). In these conditions, the electrospray phenomena are practically inhibited and the solution spray is generated only by pneumatic effects, so that the method was called direct-infusion pneumatic spray (DIPS) mass spectrometry. The previous spectrum was obtained by application of an usual capillary voltage of 3 kV and the most abundant signals at m/z 977, 949, 739, and 711 are of unknown compounds. By decreasing the spray capillary voltage to 0.5 kV, signals of the main anthocyanins in the

sample at m/z 331, 479, 493, 535, and 609 increase (marked peaks in the spectrum in the following).

The anthocyanic profiles of several red wines at different aging stages were studied by DIPS, and a great number of anthocyanins and anthocyanin-derivatives were detected. Sample preparation for analysis was performed by extraction of anthocyanins using a reverse-phase C₁₈ cartridge and recovering the analytes with methanol. In order to verify the structural assignment of the ionic species detected in the DIPS spectra, MS/MS was performed. The product ions found confirmed the presence of 15 anthocyanin-flavanol derivatives, including 11 pyranoanthocyanins, 3 vitisin A-type compounds (at m/z 531, 533, and 561, resp.) and malvin-vinyl(epi)catechin (m/z 805) (in particular in aged wines), and other several anthocyanin derivatives. The list of compounds identified is reported in Table 15.

Of course, the absolute intensity of anthocyanin signals is lower than that observed using spraying capillary voltage of 3 kV due to the lack of the ion focusing effect originating by the high voltage. This aspect reflects negatively in higher values of limit of detection (LOD) and of limit of quantification (LOQ) but positively in higher specificity of the data. DIPS fingerprint of a several years aged wine shows profound transformations of anthocyanin composition, as shown in the spectrum in Figure 21. Signals of pyranoanthocyanin and anthocyanin-flavanol derivatives arise in two m/z ranges of the spectrum, the first at m/z 579–771 and the second at m/z 751–929, respectively.

For a semiquantitative analysis, Mv-3,5-diglucoside (compound usually not present in *V. vinifera* grapes and wines) was added to the sample as internal standard (IS), and two indexes, of wine color and of wine color evolution, were calculated. The first is a total anthocyanin index of the sample. It was calculated as the sum of intensity of all anthocyanin and anthocyanin derivative signals in the TIC and was expressed as mg/L of IS. As expected, higher values of this parameter were found for nonaged wines (between 40 and 100 mg/L), instead the aged samples had anthocyanin content between 4 and 80 mg/L. In particular, very low contents were found in the two oldest samples aged in barrels

TABLE 13: Anthocyanin derivatives identified in 4–23 months aged wines.

RT (min)	Compound	[M] ⁺ (m/z)	MS ⁿ fragment ions (m/z)	$\lambda_{\max}$ (nm)	Aged wine (months)				
					4	8	13	16	23
21.7	Dp-3-glc	465	303	277, 342, 524	*	*	*	*	*
26.1	Cy-3-glc	449	287	279, 516	*	*	*	*	*
28.1	Pt-3-glc	479	317	277, 347, 525	*	*	*	*	*
34.1	Pn-3-glc	463	301	280, 517	*	*	*	*	*
35.5	Mv-3-glc	493	331	277, 348, 527	*	*	*	*	*
38.3	Dp-3-acetylglc	507	303	276, 346, 527	*	*	*	*	*
41.0	Cy-3-acetylglc	491	287	280, 523	*	*	*	*	*
41.6	Pt-3-acetylglc	521	317	270, 529	*	*	*	*	*
43.6	Pn-3-acetylglc	505	301	280, 522	*	*	*	*	*
44.3	Mv-3-acetylglc	535	331	278, 350, 530	*	*	*	*	*
43.1	Dp-3-p-coumglc <i>cis</i>	611	303	280, 301, 534	*	*	*	*	*
44.3	Dp-3-p-coumglc <i>trans</i>	611	303	282, 313, 531	*	*	*	*	*
45.1	Cy-3-p-coumglc <i>cis</i>	595	287	280, 301, 533	*	*	*	*	*
46.3	Cy-3-p-coumglc <i>trans</i>	595	287	284, 314, 524	*	*	*	*	*
45.3	Pt-3-p-coumglc <i>cis</i>	625	317	281, 301, 536	*	*	*	*	*
46.6	Pt-3-p-coumglc <i>trans</i>	625	317	282, 313, 532	*	*	*	*	*
47.5	Pn-3-p-coumglc <i>cis</i>	609	301	283, 300, 535	*	*	*	*	*
48.6	Pn-3-p-coumglc <i>trans</i>	609	301	283, 313, 526	*	*	*	*	*
47.5	Mv-3-p-coumglc <i>cis</i>	639	331	280, 301, 535	*	*	*	*	*
48.7	Mv-3-p-coumglc <i>trans</i>	639	331	282, 313, 532	*	*	*	*	*
41.1	Dp-3-cafglc <i>trans</i>	627	303	283, 331, 532	*	n.d.	n.d.	n.d.	n.d.
43.6	Pt-3-cafglc <i>trans</i>	641	317	283, 328, 531	*	*	n.d.	n.d.	n.d.
45.6	Pn-3-cafglc <i>trans</i>	625	301	283, 328, 525	*	n.d.	n.d.	n.d.	n.d.
44.8	Mv-3-cafglc <i>cis</i>	655	331		*	*	n.d.	n.d.	n.d.
45.7	Mv-3-cafglc <i>trans</i>	655	331	282, 328, 534	*	*	*	*	*
16.7	Dp-3,7-diglc	627	303	279, 523	*	n.d.	n.d.	n.d.	n.d.
20.4	Pt-3,5-diglc	641	317	275, 521	*	n.d.	n.d.	n.d.	n.d.
24.6	Pt-3,7-diglc	641	317	275, 349, 522	*	*	*	*	*
28.7	Pn-3,7-diglc	625	301		*	*	n.d.	n.d.	n.d.
23.5	Mv-3,5-diglc	655	331	275, 524	*	*	n.d.	n.d.	n.d.
30.8	Mv-3,7-diglc	655	331	278, 350, 526	*	*	*	*	*
34.7	Dp-3-glc + L(+)lactic acid	537			*	*	*	*	*
37.5	Pt-3-glc + D(−)lactic acid	551			*	*	*	*	n.d.
39.2	Pt-3-glc + L(+)lactic acid	551	317	278, 526	*	*	*	*	*
40.5	Pn-3-glc + D(−)lactic acid	535			*	*	n.d.	n.d.	n.d.
41.7	Pn-3-glc + L(+)lactic acid	535	301	281, 525	*	*	*	*	*
40.8	Mv-3-glc + D(−)lactic acid	565	331	278, 350, 530	*	*	*	*	*
42.1	Mv-3-glc + L(+)lactic acid	565	331	278, 348, 531	*	*	*	*	*
5.7	Dp-3-glc-GC	769	607, 439	531	*	*	*	*	*
7.1	Cy-3-glc-GC	753	591, 453	282, 524	*	*	*	*	*
7.2	Pt-3-glc-GC	783	621, 453	279, 532	*	*	*	*	*
10.8	Pn-3-glc-GC	767	605, 437		*	*	*	*	*
10.6	Mv-3-glc-GC	797	635, 467	281, 531	*	*	*	*	*
22.3	Mv-3-glc-EGC	797	635, 467		*	*	*	*	*
24.4	Mv-3-acetylglc-GC	839			n.d.	*	*	*	n.d.
30.9	Dp-3-p-coumglc-GC	915	607, 439		n.d.	*	*	*	n.d.
35.1	Cy-3-p-coumglc-GC	899			n.d.	*	*	n.d.	n.d.
35.4	Pt-3-p-coumglc-GC	929			n.d.	*	*	*	n.d.
38.5	Pn-3-p-coumglc-GC	913	605, 437		n.d.	*	*	*	*
38.3	Mv-3-p-coumglc-GC	943	635, 467		n.d.	*	*	*	*

TABLE 13: Continued.

RT (min)	Compound	[M] ⁺ (m/z)	MS ⁿ fragment ions (m/z)	$\lambda_{\max}$ (nm)	Aged wine (months)				
					4	8	13	16	23
41.1	Mv-3-p-coumglc-EGC	943			n.d.	*	*	*	*
10.8	Dp-3-glc-C	753	591, 439	282, 534	*	*	*	*	*
14.8	Dp-3-glc-EC	753	591, 439		*	*	*	*	*
14.9	Cy-3-glc-C	737	575, 423	286, 526	*	*	*	*	*
18.0	Cy-3-glc-EC	737	575, 423		*	*	*	*	*
16.2	Pt-3-glc-C	767	605, 453	279, 532	*	*	*	*	*
21.6	Pt-3-glc-EC	767	605, 453		*	*	*	*	*
20.3	Pn-3-glc-C	751	589, 437	283, 524	*	*	*	*	*
24.3	Pn-3-glc-EC	751	589, 437		*	*	*	*	*
21.0	Mv-3-glc-C	781	619, 467	280, 532	*	*	*	*	*
29.9	Mv-3-glc-EC	781	619, 467	279, 533	*	*	*	*	*
35.9	Mv-3-acetylglc-C	823	619, 467		n.d.	*	*	*	*
39.0	Dp-3-p-coumglc-C	899	591, 439		n.d.	*	*	*	*
40.5	Dp-3-p-coumglc-EC	899			n.d.	*	n.d.	n.d.	n.d.
39.5	Cy-3-p-coumglc-C	883			n.d.	*	*	*	n.d.
41.4	Cy-3-p-coumglc-EC	883			n.d.	*	n.d.	n.d.	n.d.
41.0	Pt-3-p-coumglc-C	913	605, 453		n.d.	*	*	*	*
42.6	Pt-3-p-coumglc-EC	913			n.d.	*	n.d.	n.d.	n.d.
41.8	Pn-3-p-coumglc-C	897	589, 437		n.d.	*	*	*	*
43.8	Pn-3-p-coumglc-EC	897			n.d.	*	n.d.	n.d.	n.d.
43.4	Mv-3-p-coumglc-C	927	619, 467	290, 538	*	*	*	*	*
46.0	Mv-3-p-coumglc-EC	927			n.d.	*	n.d.	n.d.	n.d.
35.8	Dp-3-glc-ethyl-C	781	329, 329		*	n.d.	n.d.	*	n.d.
36.7	Dp-3-glc-ethyl-EC	781			*	n.d.	n.d.	*	n.d.
39.5	Cy-3-glc-ethyl-C	765			*	n.d.	n.d.	n.d.	n.d.
39.6	Pt-3-glc-ethyl-C	795	343		*	n.d.	n.d.	*	*
42.0	Pn-3-glc-ethyl-C	779	327		*	*	n.d.	*	*
43.1	Pn-3-glc-ethyl-EC	779			*	n.d.	n.d.	*	*
41.1	Mv-3-glc-ethyl-C	809	357		*	*	*	*	*
42.2	Mv-3-glc-ethyl-C	809	357	282, 539	*	*	*	*	*
43.1	Mv-3-glc-ethyl-C	809	357	276, 537	*	*	*	*	*
45.8	Mv-3-acetylglc-ethyl-C	851	357		*	*	n.d.	n.d.	n.d.
47.4	Mv-3-p-coumglc-ethyl-C	955	357		*	*	*	*	n.d.
34.7	Dp-3-glc-ethyl-GC	797	329		*	n.d.	n.d.	n.d.	*
38.6	Cy-3-glc-ethyl-GC	781			*	n.d.	n.d.	n.d.	n.d.
38.7	Pt-3-glc-ethyl-GC	811	343		*	n.d.	n.d.	*	*
40.9	Pn-3-glc-ethyl-GC	795			*	n.d.	n.d.	n.d.	n.d.
40.6	Mv-3-glc-ethyl-GC	825	357		*	n.d.	*	*	*
41.1	Mv-3-glc-ethyl-GC	825	357	539	*	*	*	*	*
41.8	Mv-3-glc-ethyl-GC	825	357		*	n.d.	*	*	*
45.0	Mv-3-acetylglc-ethyl-GC	867			*	n.d.	n.d.	n.d.	n.d.
21.0	A-type vitisin of Dp-3-glc	533	371	297, 368, 507	n.d.	*	*	*	*
27.0	A-type vitisin of Cy-3-glc	517			n.d.	n.d.	n.d.	*	n.d.
28.7	A-type vitisin of Pt-3-glc	547	385	299, 371, 508	n.d.	*	*	*	*
35.0	A-type vitisin of Pn-3-glc	531	369	503	n.d.	n.d.	n.d.	*	*
36.0	Vitisin A	561	399	299, 372, 510	n.d.	*	*	*	*
40.7	A-type vitisin of Pt-3-p-coumglc	693			n.d.	n.d.	*	*	n.d.
43.8	A-type vitisin of Pn-3-p-coumglc	677	369	284, 508	n.d.	*	*	*	*
44.1	A-type vitisin of Mv-3-p-coumglc	707	399	271, 514	n.d.	*	*	*	*
24.4	B-type vitisin of Dp-3-glc	489	327		n.d.	*	*	*	*

TABLE 13: Continued.

RT (min)	Compound	[M] ⁺ (<i>m/z</i>)	MS ⁿ fragment ions (<i>m/z</i>)	$\lambda_{\max}$ (nm)	Aged wine (months)				
					4	8	13	16	23
33.5	B-type vitisin of Pt-3-glc	503	341	492	n.d.	*	*	*	*
38.5	B-type vitisin of Pn-3-glc	487	325		*	*	*	*	n.d.
39.5	Vitisin B	517	355	294, 358, 490	*	*	*	*	*
41.4	B-type vitisin of Pn-3-acetylglc	529	325		*	*	n.d.	n.d.	n.d.
42.4	B-type vitisin of Mv-3-acetylglc	559	355	298, 361, 494	*	*	*	*	n.d.
41.1	Acetone derivative of Pn-3-glc	501	339	475	n.d.	*	*	*	n.d.
42.1	Acetone derivative of Mv-3-glc	531	369	480	n.d.	*	*	*	*
45.5	Dp-3-glc 4-vinylphenol adduct	581	419	264, 412, 503	*	*	*	*	*
47.5	Cy-3-glc 4-vinylphenol adduct	565			n.d.	*	*	*	*
48.3	Pt-3-glc 4-vinylphenol adduct	595	433	264, 413, 502	*	*	*	*	*
50.5	Pn-3-glc 4-vinylphenol adduct	579	417	278, 406, 500	*	*	*	*	*
51.0	Mv-3-glc 4-vinylphenol adduct	609	447	263, 412, 504	*	*	*	*	*
53.2	Mv-3-acetylglc 4-vinylphenol adduct	651	447	298, 416, 505	*	*	*	*	*
49.9	Dp-3-p-coumglc 4-vinylphenol adduct	727	419		n.d.	n.d.	n.d.	*	*
52.4	Pt-3-p-coumglc 4-vinylphenol adduct	741	433	314, 504	n.d.	n.d.	n.d.	*	*
54.6	Pn-3-p-coumglc 4-vinylphenol adduct	725	417	314, 501	n.d.	n.d.	n.d.	*	*
55.2	Mv-3-p-coumglc 4-vinylphenol adduct	755	447	264, 313, 416, 505	n.d.	*	*	*	*
43.5	Dp-3-glc 4-vinylcatechol adduct	597	435	509	*	*	*	*	*
46.5	Pt-3-glc 4-vinylcatechol adduct	611	449	510	*	*	*	*	*
48.6	Pn-3-glc 4-vinylcatechol adduct	595	433	506	*	*	*	*	*
49.2	Mv-3-glc 4-vinylcatechol adduct	625	463	510	*	*	*	*	*
50.9	Mv-3-acetylglc 4-vinylcatechol adduct	667	463	513	n.d.	n.d.	*	*	*
47.6	Dp-3-p-coumglc 4-vinylcatechol adduct	743	435		*	*	*	*	*
50.5	Pt-3-p-coumglc 4-vinylcatechol adduct	757	449		n.d.	*	*	*	*
53.2	Mv-3-p-coumglc 4-vinylcatechol adduct	771	463	312, 511	*	*	*	*	*
52.0	Mv-3-glc 4-vinylguaiacol adduct	639	477	511	*	*	*	*	*
54.0	Mv-3-acetylglc 4-vinylguaiacol adduct	681	477	514	n.d.	*	*	*	*
55.7	Mv-3-p-coumglc 4-vinylguaiacol adduct	785	477	514	n.d.	*	*	*	*
49.0	Mv-3-glc 4-vinylepicatechin adduct	805			*	*	*	*	*

Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucose; p-coumglc: p-coumaroylglucoside; cafglc: caffeoylglucoside; acetylglc: acetylglucoside; catechin; GC: galocatechin; EC: epicatechin; ECG: epigallocatechin; * detected; n.d.: not detected [30].

(between 3 and 10 mg/L), inferring that anthocyanins and derivatives are undergone to severe degradation processes in barrel ageing for long time.

During aging, the chemical composition of anthocyanins (i.e., color) changes as well. Index of wine color evolution was calculated as the ratio Σ anthocyanin-derived signals intensity/total anthocyanin index and represents these chemical changes. All the nonaged wines had a value lower than 20%, while it was higher in all aged samples. As a consequence, a wine color evolution index of 20% was proposed as the limit for distinguishing between aged and nonaged wines. This index can be also correlated to the wine aging conditions, such as presence of oxidative or reductive environment (barrel or bottle), oxygen level in wine, and air exchange through the barrel staves. Moreover, because pyranoanthocyanins remain colored over a wide pH range and in the presence of sulphites [32, 85], the study of the ratio

between pyranoanthocyanins and the other anthocyanin-flavanol derivatives was proposed to predict the wine color stability.

5. Study of Grape Procyanidins by MALDI-TOF MS

Matrix-assisted laser desorption-ionization and time of flight (MALDI-TOF) mass spectrometry is a technique in which an acidic solution containing an energy-absorbing molecule (matrix) is mixed with the analyte and a highly focused laser pulses are directed to the mixture [86]. Molecules are desorbed, ionized, and accelerated by a high electrical potential, and the ions arrive to the detector in the order of their increasing *m/z* ratio. Due to robustness, tolerance to salt- and detergent-related impurities, and ability to be

TABLE 14: Molecular and fragment ions of the flavanol-anthocyanin-anthocyanin (F-A-A⁺) trimers identified in *Tempranillo* aged wines. The fragment ions are reported in order of abundance.

Proposed identity	[M] ⁺	<i>m/z</i>		
		MS ²	Fragment ions MS ³	MS ⁴
(E)C-DpG-MvG	1245	1083 [M ⁺ – 162]	921 [MS2 ⁺ – 162]	629 [MS3 ⁺ – 306]
		795 [M ⁺ – 450]	903 [MS2 ⁺ – 180]	
		921 [M ⁺ – 324]	657 [MS2 ⁺ – 426]	
		903 [M ⁺ – 342]	633 [MS2 ⁺ – 450]	
			837 [MS2 ⁺ – 246]	
(E)C-CyG-MvG	1229	1067 [M ⁺ – 162]	905 [MS2 ⁺ – 162]	
		904 [M ⁺ – 324]	917 [MS2 ⁺ – 150]	
(E)C-PtG-MvG	1259	1097 [M ⁺ – 162]	935 [MS2 ⁺ – 162]	
		971 [M ⁺ – 288]	917 [MS2 ⁺ – 180]	
		935 [M ⁺ – 324]	899 [MS2 ⁺ – 198]	
		809 [M ⁺ – 450]	671 [MS2 ⁺ – 426]	
		1079 [M ⁺ – 180]	747 [MS2 ⁺ – 350]	
(E)C-PnG-MvG	1243		971 [MS2 ⁺ – 126]	
		1081 [M ⁺ – 162]	919 [MS2 ⁺ – 162]	
(E)C-MvG-MvG	1273	1063 [M ⁺ – 180]		
		1111 [M ⁺ – 162]	949 [MS2 ⁺ – 162]	
		949 [M ⁺ – 324]	931 [MS2 ⁺ – 180]	
		661 [M ⁺ – 612]	823 [MS2 ⁺ – 288]	
		931 [M ⁺ – 342]	685 [MS2 ⁺ – 426]	
		823 [M ⁺ – 450]	661 [MS2 ⁺ – 450]	
			737 [MS2 ⁺ – 374]	
			913 [MS2 ⁺ – 198]	
			535 [MS2 ⁺ – 576]	
			331 [MS2 ⁺ – 780]	
(E)GC-DpG-MvG	1261	1099 [M ⁺ – 162]	937 [MS2 ⁺ – 162]	
(E)GC-PtG-MvG	1275	1113 [M ⁺ – 162]	951 [MS2 ⁺ – 162]	
			647 [MS2 ⁺ – 466]	
(E)GC-MvG-MvG	1289	1127 [M ⁺ – 162]	965 [MS2 ⁺ – 162]	
		965 [M ⁺ – 324]	929 [MS2 ⁺ – 198]	
		661 [M ⁺ – 628]	947 [MS2 ⁺ – 180]	
		823 [M ⁺ – 466]	823 [MS2 ⁺ – 304]	
			865 [MS2 ⁺ – 262]	
			661 [MS2 ⁺ – 466]	
			535 [MS2 ⁺ – 592]	
			839 [MS2 ⁺ – 288]	
			467 [MS2 ⁺ – 660]	
			331 [MS2 ⁺ – 796]	

MS2⁺: major fragment ion obtained in MS² analysis; MS3⁺: major fragment ion obtained in MS³ analysis; Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; G: glucose; (E)C: (epi)catechin; (E)GC: (epi)gallo catechin [60].

automated, MALDI-TOF is used to perform generation of mass map of proteins after enzymatic digestion [87]. α -cyano-4-hydroxycinnamic acid (CHCA) is the matrix commonly used for analysis of peptides and small proteins and sinapinic acid (SA) of higher molecular weight (MW) proteins (10–100 kDa). Advantages of MALDI-TOF are good mass accuracy (0.01%) and sensitivity to require very little sample for analysis.

MALDI-TOF has been used also for characterization of grape procyanidins [88–92]. LC/MS does not allow separation and identification of oligomers higher than pentamers because the separation of a large number of diastereoisomers is not possible. By operating positive-ion MALDI-TOF in the reflectron mode, flavan-3-ol oligomers and their galloylated derivatives in grape seeds extracts were studied [88]. Oligomers were detected up to heptamer as sodium adducts

TABLE 15: Compounds identified by DIPS and MS/MS in 12 wines at different aging stages.

Compound	<i>m/z</i>		Loss Da
	M^+	MS/MS ions	
Cy-glu	449	287	-162
Pn-glu	463	301	-162
Dp-glu	465	303	-162
Pt-glu	479	317	-162
Vitisin B-Pn-glu	487	325	-162
Vitisin B-Dp-glu	489	327	-162
Mv-glu	493	331	-162
Vitisin B-Pt-glu	503	341	-162
Pn-glu-Ac	505	301	-204
Dp-glu-Ac	507	303	-204
Pt-glu-Ac	521	317	-204
Vitisin A-Pn-glu	531	369	-162
Vitisin A-Dp-glu	533	371	-162
Mv-glu-Ac	535	331	-204
Vitisin A-Pt-glu	547	385	-162
Vitisin B-Mv-glu-Ac	559	355	-204
Vitisin A	561	399	-162
4-Vinylphenol-Pn-glu	579	417	-162
4-Vinylphenol-Dp-glu	581	419	-162
Cy-glu-pcoum	595	287	-308
4-Vinylphenol-Pt-glu	595	433	-162
4-Vinylcatechol-Dp-glu	597	435, 303	-162
4-Vinylphenol-Mv-glu	609	447	-162
Pn-glu-pcoum	609	301	-308
Dp-glu-pcoum	611	303	-308
4-Vinylcatechol-Mv-glu (Pinotin A)	625	463	-162
Mv-glu-pcoum	639	331	-308
4-Vinylguaiacol-Mv-glu	639	477	-162
4-Vinylphenol-Mv-glu-Ac	651	447	-204
Mv-diglu (IS)	655	493, 331	-162
4-Vinylcatechol-Mv-glu-Ac	667	463	-204
Vitisin A-Pt-glu-pcoum	693	385	-308
Vitisin A-Mv-glu-pcoum	707	399	-308
4-Vinylphenol-Pt-glu-pcoum	741	433	-308
Cat-Pn-glu	751	589	-162
Cat-Dp-glu	753	591	-162
4-Vinylphenol-Mv-glu-pcoum	755	447	-308
4-Vinylcatechol-Pt-glu-pcoum	757	449	-308
Cat-Et-Cy-glu	765	603	-162
GCat-Pn-glu or Cat-Pt-glu	767	605	-162
4-Vinylcatechol-Mv-glu-pcoum	771	463, 331	-308
Cat-Mv-glu	781	619, 467, 331	-162
GCat-Pt-glu	783	621	-162
Cat-Dp-glu-Ac	795	591, 507	-204
Cat-Et-Pt-glu	795	633, 507, 343, 317	-162
GCat-Mv-glu	797	635, 331	-162
4-VinylCat-Mv-glu	805	643	-162

TABLE 15: Continued.

Compound	M^+	m/z	MS/MS ions	Loss Da
Cat-Et-Mv-glu	809		657, 647, 357, 331	-162
Cat-Pt-glu-Ac	809		605, 519, 317	-204
GCat-Et-Pt-glu	811		649, 343	-468
Cat-Mv-glu-Ac	823		619, 535, 331	-204
GCat-Et-Mv-glu	825		663, 331	-162
GCat-Mv-glu-Ac	839		635	-204
GCat-Et-Mv-glu-Ac	867		663	-204
Cat-Pn-glu-pcoum	897		589, 301	-308
GCat-Dp-glu-pcoum	915		607	-308
Cat-Mv-glu-pcoum	927		619	-308
GCat-Pt-glu-pcoum	929		621	-308
GCat-Mv-glu-pcoum	943		635	-308
Cat-Et-Mv-glu-pcoum	955		647	-308

M^+ : molecular ion; Cat: (epi)catechin; GCat: gallo(epi)catechin; Ac: acetyl; pcoum: *p*-coumaroyl; Et: ethyl; Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glu: glucoside [70].

$[M+Na]^+$ using 2,5-dihydroxybenzoic acid (DHB) as matrix with a resolution higher than 3000, allowing to separate individual ions of different isotope composition (e.g., the ion at m/z 1177.46 was further resolved into a group of four peaks). In another study, to perform analysis of seed extracts in positive-ion reflectron mode using trans-3-indoleacrylic acid (*t*-IAA) as matrix allowed the identification of a series of compounds with MW 2 Da lower, which correspond to A-type polycatechins. Linear mode analysis provided detection of PAs oligomers sodium adducts up to nonamers [89]. The lower sensitivity of the reflectron mode for the large ions is reasonably due to their collisionally induced decomposition occurring in the flight path [88, 89]. Masses of PAs determined in both reflectron and liner modes are reported in Table 16. On the basis of the galloylated structures, the equation $290 + 288c + 152g + 23$ used to predict the mass distribution of PGPF in grape seeds was calculated (290 is the MW of the terminal catechin/epicatechin unit, *c* degree of polymerization, *g* number of galloyl esters, 23 Na atomic mass) [89].

Extraction of PAs from grape seeds for MALDI-TOF analysis was carried out with acetone/water, ethanol, or methanol/water mixtures, then a purification by extraction with ethyl acetate or chloroform which can be performed [88, 89, 91, 93].

Among the matrices tested for MALDI, DHB and *t*-IAA showed to be highly suited for PAs analysis. The experiments performed by Yang and Chien showed that DHB provides the broadest mass range and the least background noise. The dry grape seed extract was dissolved in acetone or methanol at 2 mg/mL, and a DHB matrix solution 20 mg/mL in tetrahydrofuran was prepared (the sample and matrix solutions were mixed at 1:1 v/v) [88]. Sodium apparently arises from the seeds themselves, and only a minute amount of sodium was needed. The use of DHB and water-free solvents such as anhydrous tetrahydrofuran, acetone, or

methanol for the sample and matrix preparation showed the best analytical conditions in reflectron mode.

In general tannins containing A-type bonds showed lower signal intensity with respect to the corresponding compounds with B-type bonds (between 10% and 50%). By the formula in Table 16 and the data from the literature, PAs with DP from 2 up to 11 were identified [52, 57, 89, 92].

A study of PAs in grape seeds of 35 hybrid and *V. vinifera* grape varieties is now in progress. PAs are extracted with a methanol/water 70:30 v/v solution and analyzed by MALDI-TOF in positive-ion mode using matrix DHB. PAs showed $[M+Na]^+$ adducts as main signal, and some of $[M+K]^+$ and $[M+H]^+$ adducts (Table 17). Signals of tannins containing B-type bonds and one or more A-type bonds were found in the mass spectra (Figure 22). For example, the protonated trimer was present in different forms: with two B-type bonds and MW 867 Da, with one A-type and one B-type bond and MW 865 Da, and with two A-type bonds with MW 863 Da.

6. Polyphenols and Grape Metabolomics

“Metabolomics” is the comprehensive quantitative and qualitative study of all the metabolites within a cell, tissue, or organism. The major limitation of this approach is its current inability to exhaustively describe the whole “metabolome” profile. This is due to its chemical complexity and the dynamic range limitations of most instrumental approaches. Because a single analytical technique does not provide sufficient description of whole metabolome, more methods are needed for a comprehensive view. LC/MS, direct-injection MS, and electrospray ionization (ESI) are powerful tools that offer high selectivity and sensitivity, allowing detection of nonvolatile and labile components in the extract. The coupling of the techniques with GC/MS analysis of volatile compounds, in general, provides a sufficiently wide panorama of the sample metabolomics. The selection of the

TABLE 16: Masses observed by MALDI-TOF analysis calculated using the equation $290 + 288c + 152g + 23$ (290: MW of the terminal catechin unit; c : degree of polymerization; g : number of galloyl ester; 23: Na atomic mass). n.d.: not observed [88].

Polymer	no. galloyl ester	Calculated $[M + Na]^+$	Observed $[M + Na]^+$	
			Positive linear	Positive reflectron
Dimer	0	601	600	601
	1	753	752	753
	2	905	905	905
Trimer	0	889	889	889
	1	1041	1041	1041
	2	1193	1193	1193
	3	1345	1346	1345
Tetramer	0	1177	1177	1177
	1	1329	1329	1329
	2	1481	1482	1482
	3	1634	1634	1634
	4	1786	1785	1786
Pentamer	0	1466	1465	1466
	1	1618	1618	1618
	2	1770	1770	1770
	3	1922	1922	1922
	4	2074	2074	2074
	5	2226	n.d.	n.d.
Hexamer	0	1754	1754	1754
	1	1906	1907	1906
	2	2058	2059	2058
	3	2210	2211	n.d.
	4	2362	2362	2362
	5	2514	2513	n.d.
	6	2666	2667	n.d.
Heptamer	0	2042	2043	2042
	1	2194	2195	2194
	2	2346	2346	2346
	3	2398	2499	2499
	4	2651	2651	n.d.
	5	2803	2800	n.d.
	6	2955	2954	n.d.
	7	3107	n.d.	n.d.
Octamer	0	2330	2330	2330
	1	2483	2483	2483
	2	2635	2635	n.d.
	3	2787	2787	n.d.
	4	2939	2938	n.d.
	5	3091	3090	n.d.
	6	3243	n.d.	n.d.
Nonamer	0	2619	2618	2618
	1	2771	2770	n.d.
	2	2923	2923	n.d.
	3	3075	3075	n.d.
	4	3227	n.d.	n.d.
Decamer	0	2907	2907	n.d.
	1	3059	3060	n.d.
	2	3211	3212	n.d.
	3	3363	n.d.	n.d.
Undecamer	0	3195	3194	n.d.
	1	3347	3349	n.d.

TABLE 17: Main PAs identified in the MALDI-TOF positive-ion spectra of seeds extracts of 35 hybrid and *V. vinifera* grape varieties. Signals were divided in A and B series in reference to the interflavanic bond type: series B only B-type interflavanic bonds, and also A-type bonds are included in series A. Signals of sodium and protonated ions are reported. G: galloyl ester.

Procyanidin	Series B		Series A	
	[M + H] ⁺	[M + Na] ⁺	[M + H] ⁺	[M + Na] ⁺
Dimer A-type			577	
Dimer B-type		601		
Dimer, 1G		753		
Trimer	867			863, 865
Trimer		889		887
Dimer, 2G		905		903
Trimer, 1G		1041		1039
Tetramer		1177		1175
Trimer, 2G		1193		1191
Tetramer, 1G	1305	1329	1303	1327
Trimer, 3G		1345		1343
Pentamer		1465		1463
Tetramer, 2G		1481		1629, 1631
Pentamer, 1G		1617		1613, 1615
Tetramer, 3G		1633		1629, 1631
Hexamer		1753		1751
Pentamer, 2G		1769		1765, 1767
Tetramer, 4G		1785		1781, 1783
Hexamer, 1G		1905		1903
Pentamer, 3G		1921		1917, 1919
Heptamer		2041		
Hexamer, 2G		2057		2053, 2055
Pentamer, 4G		2073		2069, 2071
Heptamer, 1G		2193		2189, 2191
Hexamer, 3G		2209		2205, 2207
Pentamer, 5G		2225		2221, 2223
Heptamer, 2G		2345		2341, 2343
Hexamer, 4G		2361		2357, 2359
Heptamer, 3G		2497		2495
Hexamer, 5G		2513		2511
Octamer, 2G		2633		2631
Heptamer, 4G		2649		2647
Hexamer, 6G		2665		2663
Octamer, 3G		2785		2783
Heptamer 5G		2802		
Nonamer, 2G		2922		2919
Octamer, 4G		2937		2935
Heptamer, 6G		2953		2951
Nonamer, 3G		3073		3071
Octamer, 5G		3089		3087
Decamer, 2G		3209		3207
Nonamer, 4G		3225		3223
Octamer, 6G		3241		
Decamer, 3G		3361		
Nonamer, 5G		3377		3376
Undecamer, 2G		3496		

most suitable techniques is generally a compromise between speed, selectivity, and sensitivity. Due to the complexity of plant extracts, statistical multivariate analysis of data (principal component analysis and cluster analysis) has to be

performed to ensure good analytical rigorousness and define both similarities and differences among samples [94].

In general, an “untargeted” metabolomics approach provides sensitivity, resolution, and high-throughput capacity and identification of thousands compounds in a single run [95]. Several studies reported that by performing a “targeted” analysis of specific metabolites, large part of the molecular information regarding the metabolome of complex samples (e.g., the wine) is missed [96, 97]. On the other hand, other several studies performed in target analysis provided interesting results in the wine study [98–100]. For example, in targeted analysis of red wines, anthocyanins and the pigments formed during wine ageing were reported as main biomarkers [95].

A middle-way method between these two approaches is the “suspects screening analysis.” In this study the identification of metabolites relies on available specific information on compounds such as their molecular formula and structure [101]. This approach is applied in our laboratories to the study of metabolomics of grape varieties. Grape berries are powered using liquid nitrogen (in order to minimize possible artifacts) and extracted with methanol. An internal standard is added, and the extract is analyzed with a LC/QTOF system with nominal resolution 40.000 which provides accurate mass measurements.

A library called *Grape Metabolomics* is actually under construction. This database includes the information available in the literature and found in electronic databases on the potential grape metabolites. Partial confirmation of the library hits was achieved by performing the identification of metabolites in extracts of some grape varieties taken as models for the peculiar chemical characteristics previously studied (e.g., *Raboso Piave* for the study of anthocyanins and polyphenols and *Moscato Bianco* for aroma precursors) [102, 103]. Currently, this library contains around 1000 putative compounds of grape with MW between 100 and 1700 Da. When data processing of a sample provides identification of a new compound with score sufficiently confident, it is added to the library. As a consequence, a further increase of the library will be possible. Compounds are identified on the basis of accurate mass measurements and their isotope patterns, and the identification is confirmed by MS/MS (multiple mass spectrometry).

With this approach, between 260 and 450 signals were assigned to putative phenolic compounds in grapes (the number depending on the grape variety), mainly including nutraceutical and antioxidant compounds such as anthocyanins, flavones and flavanones, flavanols and procyanidins, phytoalexins, and phenolic acids. Average 30–60 hits had an identification score higher than 99% and a hundred higher 95% (Table 18). For example, in analysis of *Raboso Piave* grape extract, 17 stilbenes and derivatives were identified, among them *trans*-resveratrol, piceatannol, *cis*- and *trans*-piceid, several viniferins, and resveratrol dimers, trimers, and tetramers. Moreover, tentative of identification of a number of aroma precursors (mono- and diterpenols glycoside, norisoprenoids), primary metabolites and peptides, is now in progress.

TABLE 18: Phenolic compounds identified by LC/QTOF mass spectrometry and “suspects screening” analysis using the library *GrapeMetabolomics* in the study of metabolomics of *Raboso Piave* grape extract. The compounds identified with identification score (id.) higher than 95% are reported.

Phenolic compound	Id. score	Exact mass	Formula
Vanillic acid	99.9	168.0423	C ₈ H ₈ O ₄
cis-piceid	99.9	390.1315	C ₂₀ H ₂₂ O ₈
Pentahydroxyflavone A	99.8	302.0427	C ₁₅ H ₁₀ O ₇
Myricetin-3-O-rhamnoside	99.8	464.0955	C ₂₁ H ₂₀ O ₁₂
(epi)catechin gallate	99.8	442.0900	C ₂₂ H ₁₈ O ₁₀
Hydroxycoumarin	99.8	162.0317	C ₉ H ₆ O ₃
Rhamnetin	99.8	316.0583	C ₁₆ H ₁₂ O ₇
trans-resveratrol	99.8	228.0786	C ₁₄ H ₁₂ O ₃
Myricetin	99.8	318.0376	C ₁₅ H ₁₀ O ₈
Coumarin	99.8	146.0368	C ₉ H ₆ O ₂
Resveratrol tetramer 3	99.8	906.2676	C ₅₆ H ₄₂ O ₁₂
Pentahydroxyflavone B	99.7	302.0427	C ₁₅ H ₁₀ O ₇
Pyrogallol	99.7	126.0317	C ₆ H ₆ O ₃
Syringetin	99.7	346.0689	C ₁₇ H ₁₄ O ₈
Taxifolin-deoxy hexoside B	99.7	450.1162	C ₂₁ H ₂₂ O ₁₁
Gingerol	99.7	294.1831	C ₁₇ H ₂₆ O ₄
Dimethoxyphenol	99.6	154.0630	C ₈ H ₁₀ O ₃
Barbaloin	99.6	418.1264	C ₂₁ H ₂₂ O ₉
trans-caffeoyl-tartaric acid	99.6	312.0481	C ₁₃ H ₁₂ O ₉
Ferulic acid	99.6	194.0579	C ₁₀ H ₁₀ O ₄
Delphinidin p-coumarylmonoglucoside	99.6	611.1401	C ₃₀ H ₂₇ O ₁₄
Laricitrin-3-O-glucoside	99.6	494.1060	C ₂₂ H ₂₂ O ₁₃
Emodin 8-glucoside	99.5	432.1057	C ₂₁ H ₂₀ O ₁₀
<i>E</i> - ϵ -viniferin	99.5	454.1416	C ₂₈ H ₂₂ O ₆
Isorhamnetin-3-O-glucoside	99.5	478.1111	C ₂₂ H ₂₂ O ₁₂
Laricitrin	99.4	332.0532	C ₁₆ H ₁₂ O ₈
Cyanidin p-coumarylmonoglucoside	99.4	595.1452	C ₃₀ H ₂₇ O ₁₃
Esahydroxyflavanone-C-deoxy hexoside	99.4	466.1111	C ₂₁ H ₂₂ O ₁₂
Pentahydroxyflavanone-C-hexoside	99.4	466.1111	C ₂₁ H ₂₂ O ₁₂
Kaempferol-deoxy hexoside	99.4	432.1056	C ₂₁ H ₂₀ O ₁₀
<i>Z</i> - ϵ -viniferin	99.4	454.1416	C ₂₈ H ₂₂ O ₆
(epi)galocatechin-catechin	99.3	594.1373	C ₃₀ H ₂₆ O ₁₃
Caffeic acid	99.3	180.0423	C ₉ H ₈ O ₄
Taxifolin-deoxy hexoside A	99.3	450.1162	C ₂₁ H ₂₂ O ₁₁
Taxifolin-pentoside B	99.3	436.1006	C ₂₀ H ₂₀ O ₁₁
Guaiacol	99.2	124.0524	C ₇ H ₈ O ₂
Resveratrol dimer 4	99.2	454.1416	C ₂₈ H ₂₂ O ₆
Methylnaringenin	99.2	286.0841	C ₁₆ H ₁₄ O ₅
Petunidin-3-O-monoglucoside	99.2	479.1190	C ₂₂ H ₂₃ O ₁₂
Tamarixetin	99.2	316.0583	C ₁₆ H ₁₂ O ₇
Peonidin acetylmonoglucoside	99.2	505.1346	C ₂₄ H ₂₅ O ₁₂
Dihydroquercetin	99.2	304.0583	C ₁₅ H ₁₂ O ₇
Syringetin-3-O-glucoside	99.1	508.1217	C ₂₃ H ₂₄ O ₁₃
Quercetin-3-O-rhamnoside	99.1	448.1006	C ₂₁ H ₂₀ O ₁₁
Fisetin	99.1	286.0477	C ₁₅ H ₁₀ O ₆
Luteolin	99.1	286.0477	C ₁₅ H ₁₀ O ₆
Vanillic acid beta-D-glucopyranoside	98.9	330.0951	C ₁₄ H ₁₈ O ₉
Peonidin p-coumaroylmonoglucoside	98.9	609.1608	C ₃₁ H ₂₉ O ₁₃

TABLE 18: Continued.

Phenolic compound	Id. score	Exact mass	Formula
Piceatannol	98.7	244.0736	C ₁₄ H ₁₂ O ₄
Petunidin acetylmonoglucoside	98.6	521.1295	C ₂₄ H ₂₅ O ₁₃
Myricetin-3-O-glucoside	98.5	480.0904	C ₂₁ H ₂₀ O ₁₃
p-hydroxybenzoic acid	98.4	138.0317	C ₇ H ₆ O ₃
Methylsyringate beta-D-glucopyranoside	98.3	374.1213	C ₁₆ H ₂₂ O ₁₀
Quercetin-3-O-glucoside	98.0	464.0955	C ₂₁ H ₂₀ O ₁₂
Resveratrol dimer 3	98.0	454.1416	C ₂₈ H ₂₂ O ₆
Silybin	97.9	482.1213	C ₂₅ H ₂₂ O ₁₀
Methylvanillate beta-D-glucopyranoside	97.9	344.1107	C ₁₅ H ₂₀ O ₉
Syringaldehyde 4-O-beta-D-glucopyranoside	97.9	344.1107	C ₁₅ H ₂₀ O ₉
Procyanidin (B1, B2, B3, B4, B5)	97.8	578.1424	C ₃₀ H ₂₆ O ₁₂
Tetrahydroxy-dimethoxyflavanone-hexoside	97.8	510.1373	C ₂₃ H ₂₆ O ₁₃
Malvidin-3-O-monoglucoside	97.7	493.1346	C ₂₃ H ₂₅ O ₁₂
Resveratrol trimer 1	97.7	680.2046	C ₄₂ H ₃₂ O ₉
Pelargonidin p-coumaroylmonoglucoside	97.6	579.1503	C ₃₀ H ₂₇ O ₁₂
Cyanidin-3-O-monoglucoside	97.6	449.1084	C ₂₁ H ₂₁ O ₁₁
Resveratrol trimer 2	97.5	680.2046	C ₄₂ H ₃₂ O ₉
Di-O-methylquercetin B	97.5	330.0740	C ₁₇ H ₁₄ O ₇
p-cumaric acid beta-D-glucopyranoside	97.5	326.1002	C ₁₅ H ₁₈ O ₈
Delphinidin-3-O-monoglucoside	97.4	465.1033	C ₂₁ H ₂₁ O ₁₂
Quercetin-3-O-glucuronide	97.4	478.0747	C ₂₁ H ₁₈ O ₁₃
p-cumaric acid	97.2	164.0473	C ₉ H ₈ O ₃
Delphinidin acetylmonoglucoside	97.2	507.1139	C ₂₃ H ₂₃ O ₁₃
Procyanidin (T2, T3, T4)	97.0	866.2058	C ₄₅ H ₃₈ O ₁₈
Petunidin p-coumaroylmonoglucoside	96.8	625.1557	C ₃₁ H ₂₉ O ₁₄
Malvidin acetylmonoglucoside	96.7	535.1452	C ₂₅ H ₂₇ O ₁₃
Di-O-methylquercetin A	96.5	330.0740	C ₁₇ H ₁₄ O ₇
Malvidin p-coumaroylmonoglucoside	96.3	639.1714	C ₃₂ H ₃₁ O ₁₄
Methylgallic acid isomer 2	96.0	184.0372	C ₈ H ₈ O ₅
Resveratrol dimer 5	95.5	454.1416	C ₂₈ H ₂₂ O ₆
Coniferaldehyde 4-O-beta-D-glucopyranoside	95.3	340.1158	C ₁₆ H ₂₀ O ₈
Myricetin-3-O-glucuronide	95.0	494.0697	C ₂₁ H ₁₈ O ₁₄

7. Conclusions

Mass spectrometry plays a very important role for research and quality control in the viticulture and oenology field. The soft ionization conditions of LC/MS and the minor sample purification usually needed make these techniques more suitable to study the structures of polyphenols and anthocyanins in grape extracts and for the study of structures correlated to the color changing of red wines. These methods also allow to characterize the high-MW compounds of grape, such as procyanidins, proanthocyanidins, prodelphinidins, and tannins. The important role of LC/MS in the structural study of polyphenols is also confirmed by the considerable number of papers appeared in the literature in the last years.

Complementary use of different MS techniques can be highly effective to characterize the large panorama of compounds of grape and wine. For example, the use of LC/MS, MALDI-TOF and MS/MS techniques allowed the characterization of procyanidin oligomers up to dodecamers;

the coupling of LC/MS with MS/MS techniques is very effective in particular for characterization of glycoside compounds, and GC/MS and LC/MS analyses allow the characterization of hundreds volatile and nonvolatile compounds providing practically the whole metabolome of grape. Further development of these metabolomic approaches will provide effective tools for identification of a high number of important compounds in grape with few analyses and minimal sample preparation, providing useful information on the compounds involved in the metabolisms of cells and tissues.

Acknowledgments

The author would like to thank Dr. Laura Molin and Dr. Roberta Seraglia for the MALDI-TOF data of PAs and for the spectrum of Cabernet Sauvignon grape seeds extract kindly provided.

References

- [1] S. Renaud and M. De Lorgeril, "Wine, alcohol, platelets, and the French paradox for coronary heart disease," *The Lancet*, vol. 339, no. 8808, pp. 1523–1526, 1992.
- [2] V. Cheynier and J. Rigaud, "HPLC separation and characterization of flavonols in the skin of *Vitis vinifera* var. Cinsault," *American Journal of Enology and Viticulture*, vol. 37, no. 4, pp. 248–252, 1986.
- [3] S. Vidal, L. Francis, S. Guyot et al., "The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium," *Journal of the Science of Food and Agriculture*, vol. 83, no. 6, pp. 564–573, 2003.
- [4] R. Flamini, "Mass spectrometry in grape and wine chemistry. Part I: polyphenols," *Mass Spectrometry Reviews*, vol. 22, no. 4, pp. 218–250, 2003.
- [5] N. Castillo-Muñoz, S. Gómez-Alonso, E. García-Romero, and I. Hermosín-Gutiérrez, "Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 3, pp. 992–1002, 2007.
- [6] N. Castillo-Muñoz, S. Gómez-Alonso, E. García-Romero, M. V. Gómez, A. H. Velders, and I. Hermosín-Gutiérrez, "Flavonol 3-O-glycosides series of *Vitis vinifera* Cv. Petit Verdot red wine grapes," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 1, pp. 209–219, 2009.
- [7] M. Jang, L. Cai, G. O. Udeani et al., "Cancer chemopreventive activity of resveratrol, a natural product derived from grapes," *Science*, vol. 275, no. 5297, pp. 218–220, 1997.
- [8] L. M. Hung, J. K. Chen, S. S. Huang, R. S. Lee, and M. J. Su, "Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes," *Cardiovascular Research*, vol. 47, no. 3, pp. 549–555, 2000.
- [9] E. N. Frankel, A. L. Waterhouse, and J. E. Kinsella, "Inhibition of human LDL oxidation by resveratrol," *The Lancet*, vol. 341, no. 8852, pp. 1103–1104, 1993.
- [10] L. Frémont, L. Belguendouz, and S. Delpal, "Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids," *Life Sciences*, vol. 64, no. 26, pp. 2511–2521, 1999.
- [11] A. A. E. Bertelli, L. Giovannini, D. Giannessi et al., "Antiplatelet activity of synthetic and natural resveratrol in red wine," *International Journal of Tissue Reactions*, vol. 17, no. 1, pp. 1–3, 1995.
- [12] C. R. Pace-Asciak, S. Hahn, E. P. Diamandis, G. Soleas, and D. M. Goldberg, "The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease," *Clinica Chimica Acta*, vol. 235, no. 2, pp. 207–219, 1995.
- [13] L. Bavaresco, F. Mattivi, M. De Rosso, and R. Flamini, "Effects of elicitors, viticultural factors, and enological practices on resveratrol and stilbenes in grapevine and wine," *Mini-Reviews in Medicinal Chemistry*, vol. 12, no. 13, pp. 1366–1381, 2012.
- [14] L. Bavaresco, E. Cantù, M. Fregoni, and M. Trevisan, "Constitutive stilbene contents of grapevine cluster stems as potential source of resveratrol in wine," *Vitis*, vol. 36, no. 3, pp. 115–118, 1997.
- [15] P. Waffo-Teguo, D. Lee, M. Cuendet, J. M. Mèrillon, J. M. Pezzuto, and A. Douglas Kinghorn, "Two new stilbene dimer glucosides from grape (*Vitis vinifera*) cell cultures," *Journal of Natural Products*, vol. 64, no. 1, pp. 136–138, 2001.
- [16] R. H. Cichewicz, S. A. Kouzi, and M. T. Hamann, "Dimerization of resveratrol by the grapevine pathogen *Botrytis cinerea*," *Journal of Natural Products*, vol. 63, no. 1, pp. 29–33, 2000.
- [17] M. Sbaghi, P. Jeandet, R. Bessis, and P. Leroux, "Degradation of stilbene-type phytoalexins in relation to the pathogenicity of *Botrytis cinerea* to grapevines," *Plant Pathology*, vol. 45, no. 1, pp. 139–144, 1996.
- [18] T. C. Somers, "The polymeric nature of wine pigments," *Phytochemistry*, vol. 10, no. 9, pp. 2175–2186, 1971.
- [19] C. F. Timberlake and P. Bridle, "Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines," *American Journal of Enology and Viticulture*, vol. 27, no. 3, pp. 97–105, 1976.
- [20] H. Fulcrand, C. Benabdeljalil, J. Rigaud, V. Cheynier, and M. Moutounet, "A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins," *Phytochemistry*, vol. 47, no. 7, pp. 1401–1407, 1998.
- [21] Y. Hayasaka and R. E. Asenstorfer, "Screening for potential pigments derived from anthocyanins in red wine using nano-electrospray tandem mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 4, pp. 756–761, 2002.
- [22] C. Alcalde-Eon, M. T. Escribano-Bailón, C. Santos-Buelga, and J. C. Rivas-Gonzalo, "Separation of pyranoanthocyanins from red wine by column chromatography," *Analytica Chimica Acta*, vol. 513, no. 1, pp. 305–318, 2004.
- [23] J. L. Gómez-Ariza, T. García-Barrera, and F. Lorenzo, "Anthocyanins profile as fingerprint of wines using atmospheric pressure photoionisation coupled to quadrupole time-of-flight mass spectrometry," *Analytica Chimica Acta*, vol. 570, no. 1, pp. 101–108, 2006.
- [24] H. Wang, E. J. Race, and A. J. Shrikhande, "Characterization of anthocyanins in grape juices by ion trap liquid chromatography-mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 7, pp. 1839–1844, 2003.
- [25] L. Stella, M. De Rosso, A. Panighel, A. Dalla Vedova, R. Flamini, and P. Traldi, "Collisionally induced fragmentation of $[M-H]^+$ species of resveratrol and piceatannol investigated by deuterium labelling and accurate mass measurements," *Rapid Communications in Mass Spectrometry*, vol. 22, no. 23, pp. 3867–3872, 2008.
- [26] M. E. Camire, A. Chaovanalikit, M. P. Dougherty, and J. Briggs, "Blueberry and grape anthocyanins as breakfast cereal colorants," *Journal of Food Science*, vol. 67, no. 1, pp. 438–441, 2002.
- [27] J. He and M. M. Giusti, "Anthocyanins: natural colorants with health-promoting properties," *Annual Review of Food Science and Technology*, vol. 1, pp. 163–187, 2010.
- [28] V. Hong and R. E. Wrolstad, "Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection," *Journal of Agricultural and Food Chemistry*, vol. 38, no. 3, pp. 698–708, 1990.
- [29] A. Versari, R. B. Boulton, and G. P. Parpinello, "A comparison of analytical methods for measuring the color components of red wines," *Food Chemistry*, vol. 106, no. 1, pp. 397–402, 2008.
- [30] C. Alcalde-Eon, M. T. Escribano-Bailón, C. Santos-Buelga, and J. C. Rivas-Gonzalo, "Changes in the detailed pigment composition of red wine during maturity and ageing: a comprehensive study," *Analytica Chimica Acta*, vol. 563, no. 1–2, pp. 238–254, 2006.
- [31] C. Boido, F. Alcalde-Eon, E. Carrau, E. Dellacassa, and J. C. Rivas-Gonzalo, "Aging effect on the pigment composition and

- color of *Vitis vinifera* L. cv. Tannat wines. Contribution of the main pigment families to wine color," *Journal of Agricultural and Food Chemistry*, vol. 54, pp. 6692–6704, 2006.
- [32] J. Bakker and C. F. Timberlake, "Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines," *Journal of Agricultural and Food Chemistry*, vol. 45, no. 1, pp. 35–43, 1997.
- [33] R. E. Asenstorfer, Y. Hayasaka, and G. P. Jones, "Isolation and structures of oligomeric wine pigments by bisulfite-mediated ion-exchange chromatography," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 12, pp. 5957–5963, 2001.
- [34] M. Schwarz, T. C. Wabnitz, and P. Winterhalter, "Pathway leading to the formation of anthocyanin-vinylphenol adducts and related pigments in red wines," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 12, pp. 3682–3687, 2003.
- [35] A. Morata, F. Calderón, M. C. González, M. C. Gómez-Cordovés, and J. A. Suárez, "Formation of the highly stable pyranoanthocyanins (vitisins A and B) in red wines by the addition of pyruvic acid and acetaldehyde," *Food Chemistry*, vol. 100, no. 3, pp. 1144–1152, 2007.
- [36] S. Pati, I. Losito, G. Gambacorta, E. La Notte, F. Palmisano, and P. G. Zambonin, "Simultaneous separation and identification of oligomeric procyanidins and anthocyanin-derived pigments in raw red wine by HPLC-UV-ESI-MSn," *Journal of Mass Spectrometry*, vol. 41, no. 7, pp. 861–871, 2006.
- [37] W. M. A. Niessen and A. P. Tinke, "Liquid chromatography-mass spectrometry. General principles and instrumentation," *Journal of Chromatography A*, vol. 703, no. 1–2, pp. 37–57, 1995.
- [38] E. de Hoffmann, "Tandem mass spectrometry: a primer," *Journal of Mass Spectrometry*, vol. 31, no. 2, pp. 125–137, 1996.
- [39] J. Abian, "The coupling of gas and liquid chromatography with mass spectrometry," *Journal of Mass Spectrometry*, vol. 34, no. 3, pp. 157–168, 1999.
- [40] R. Flamini, A. Dalla Vedova, D. Cancian, A. Panighel, and M. De Rosso, "GC/MS-positive ion chemical ionization and MS/MS study of volatile benzene compounds in five different woods used in barrel making," *Journal of Mass Spectrometry*, vol. 42, no. 5, pp. 641–646, 2007.
- [41] H. J. Li and M. L. Deinzer, "Tandem mass spectrometry for sequencing proanthocyanidins," *Analytical Chemistry*, vol. 79, no. 4, pp. 1739–1748, 2007.
- [42] M. D. Rosso, A. Panighel, A. D. Vedova, L. Stella, and R. Flamini, "Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 5, pp. 1915–1920, 2009.
- [43] B. Sun, M. C. Leandro, V. de Freitas, and M. I. Spranger, "Fractionation of red wine polyphenols by solid-phase extraction and liquid chromatography," *Journal of Chromatography A*, vol. 1128, no. 1–2, pp. 27–38, 2006.
- [44] S. González-Manzano, C. Santos-Buelga, J. J. Pérez-Alonso, J. C. Rivas-Gonzalo, and M. T. Escribano-Bailón, "Characterization of the mean degree of polymerization of proanthocyanidins in red wines using liquid chromatography-mass spectrometry (LC-MS)," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 12, pp. 4326–4332, 2006.
- [45] B. Gabetta, N. Fuzzati, A. Griffini et al., "Characterization of proanthocyanidins from grape seeds," *Fitoterapia*, vol. 71, no. 2, pp. 162–175, 2000.
- [46] S. A. Lazarus, G. E. Adamson, J. F. Hammerstone, and H. H. Schmitz, "High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 9, pp. 3693–3701, 1999.
- [47] V. Núñez, C. Gómez-Cordovés, B. Bartolomé, Y. J. Hong, and A. E. Mitchell, "Non-galloylated and galloylated proanthocyanidin oligomers in grape seeds from *Vitis vinifera* L. cv. Graciano, Tempranillo and Cabernet Sauvignon," *Journal of the Science of Food and Agriculture*, vol. 86, no. 6, pp. 915–921, 2006.
- [48] Q. Wu, M. Wang, and J. E. Simon, "Determination of proanthocyanidins in fresh grapes and grape products using liquid chromatography with mass spectrometric detection," *Rapid Communications in Mass Spectrometry*, vol. 19, no. 14, pp. 2062–2068, 2005.
- [49] R. Flamini, M. Rosso De, A. Smaniotto et al., "Fast analysis of isobaric grape anthocyanins by chip-liquid chromatography/mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 23, no. 18, pp. 2891–2896, 2009.
- [50] Y. Hayasaka, E. J. Waters, V. Cheynier, M. J. Herderich, and S. Vidal, "Characterization of proanthocyanidins in grape seeds using electrospray mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 17, no. 1, pp. 9–16, 2003.
- [51] V. Cheynier, T. Doco, H. Fulcrand et al., "ESI-MS analysis of polyphenolic oligomers and polymers," *Analisis*, vol. 25, no. 8, pp. M32–M37, 1997.
- [52] C. P. Passos, S. M. Cardoso, M. R. M. Domingues, P. Domingues, C. M. Silva, and M. A. Coimbra, "Evidence for galloylated type-A procyanidins in grape seeds," *Food Chemistry*, vol. 105, no. 4, pp. 1457–1467, 2007.
- [53] A. Delcambre and C. Saucier, "Identification of new flavan-3-ol monoglycosides by UHPLC-ESI-Q-TOF in grapes and wine," *Journal of Mass Spectrometry*, vol. 47, no. 6, pp. 727–736, 2012.
- [54] M. Monagas, R. Suárez, C. Gómez-Cordovés, and B. Bartolomé, "Simultaneous determination of nonanthocyanin phenolic compounds in red wines by HPLC-DAD/ESI-MS," *American Journal of Enology and Viticulture*, vol. 56, no. 2, pp. 139–147, 2005.
- [55] H. Fulcrand, S. Remy, J. M. Souquet, V. Cheynier, and M. Moutounet, "Study of wine tannin oligomers by on-line liquid chromatography electrospray ionization mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 3, pp. 1023–1028, 1999.
- [56] S. Vidal, E. Meudec, V. Cheynier, G. Skouroumounis, and Y. Hayasaka, "Mass spectrometric evidence for the existence of oligomeric anthocyanins in grape skins," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 23, pp. 7144–7151, 2004.
- [57] I. I. Rockenbach, E. Junfer, C. Ritter et al., "Characterization of flavan-3-ols in seeds of grape pomace by CE, HPLC-DAD-MSn and LC-ESI-FTICR-MS," *Food Research International*, vol. 48, no. 2, pp. 845–855, 2012.
- [58] M. De Rosso, L. Tonidandel, R. Larcher et al., "Study of anthocyanic profiles of twenty-one hybrid grape varieties by liquid chromatography and precursor-Ion mass spectrometry," *Analytica Chimica Acta*, vol. 732, pp. 120–129, 2012.
- [59] S. Pati, M. T. Liberatore, G. Gambacorta, D. Antonacci, and E. La Notte, "Rapid screening for anthocyanins and anthocyanin dimers in crude grape extracts by high performance liquid chromatography coupled with diode array detection and tandem mass spectrometry," *Journal of Chromatography A*, vol. 1216, no. 18, pp. 3864–3868, 2009.
- [60] C. Alcalde-Eon, M. T. Escribano-Bailón, C. Santos-Buelga, and J. C. Rivas-Gonzalo, "Identification of dimeric anthocyanins

- and new oligomeric pigments in red wine by means of HPLC-DAD-ESI/MSn," *Journal of Mass Spectrometry*, vol. 42, no. 6, pp. 735–748, 2007.
- [61] L. Hartmanova, V. Ranc, B. Papouskova, P. Bednar, V. Havlicek, and K. Lemr, "Fast profiling of anthocyanins in wine by desorption nano-electrospray ionization mass spectrometry," *Journal of Chromatography A*, vol. 1217, no. 25, pp. 4223–4228, 2010.
- [62] B. Sun, T. A. Fernandes, and M. I. Spranger, "A new class of anthocyanin-procyanidin condensation products detected in red wine by electrospray ionization multi-stage mass spectrometry analysis," *Rapid Communications in Mass Spectrometry*, vol. 24, no. 3, pp. 254–260, 2010.
- [63] B. Papoušková, P. Bednář, K. Hron et al., "Advanced liquid chromatography/mass spectrometry profiling of anthocyanins in relation to set of red wine varieties certified in Czech Republic," *Journal of Chromatography A*, vol. 1218, no. 42, pp. 7581–7591, 2011.
- [64] E. Revilla, J. M. Ryan, and G. Martín-Ortega, "Comparison of several procedures used for the extraction of anthocyanins from red grapes," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 11, pp. 4592–4597, 1998.
- [65] L. Gao, B. Girard, G. Mazza, and A. G. Reynolds, "Changes in anthocyanins and color characteristics of Pinot Noir during different vinification processes," *Journal of Agricultural and Food Chemistry*, vol. 45, no. 6, pp. 2003–2008, 1997.
- [66] D. Favretto and R. Flamini, "Application of electrospray ionization mass spectrometry to the study of grape anthocyanins," *American Journal of Enology and Viticulture*, vol. 51, no. 1, pp. 55–64, 2000.
- [67] E. Salas, V. Atanasova, C. Poncet-Legrand, E. Meudec, J. P. Mazauric, and V. Cheynier, "Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions," *Analytica Chimica Acta*, vol. 513, no. 1, pp. 325–332, 2004.
- [68] B. Sun, C. P. Reis Santos, M. C. Leandro, V. De Freitas, and M. I. Spranger, "High-performance liquid chromatography/electrospray ionization mass spectrometric characterization of new products formed by the reaction between flavanols and malvidin 3-glucoside in the presence of acetaldehyde," *Rapid Communications in Mass Spectrometry*, vol. 21, no. 14, pp. 2227–2236, 2007.
- [69] P. Mazzuca, P. Ferranti, G. Picariello, L. Chianese, and F. Addeo, "Mass spectrometry in the study of anthocyanins and their derivatives: differentiation of *Vitis vinifera* and hybrid grapes by liquid chromatography/electrospray ionization mass spectrometry and tandem mass spectrometry," *Journal of Mass Spectrometry*, vol. 40, no. 1, pp. 83–90, 2005.
- [70] R. Flamini, F. Agnolin, R. Seraglia et al., "A fast and selective method for anthocyanic profiling of red wines by direct-infusion pneumatic spray mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 26, no. 3, pp. 355–362, 2012.
- [71] S. Vidal, L. Francis, A. Noble, M. Kwiatkowski, V. Cheynier, and E. Waters, "Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine," *Analytica Chimica Acta*, vol. 513, no. 1, pp. 57–65, 2004.
- [72] C. Dufour and I. Sauvaitre, "Interactions between anthocyanins and aroma substances in a model system. Effect on the flavor of grape-derived beverages," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 5, pp. 1784–1788, 2000.
- [73] M. Garcia-Alonso, G. Rimbach, M. Sasai et al., "Electron spin resonance spectroscopy studies on the free radical scavenging activity of wine anthocyanins and pyranoanthocyanins," *Molecular Nutrition and Food Research*, vol. 49, no. 12, pp. 1112–1119, 2005.
- [74] I. Tedesco, G. Luigi Russo, F. Nazzaro, M. Russo, and R. Palumbo, "Antioxidant effect of red wine anthocyanins in normal and catalase-inactive human erythrocytes," *Journal of Nutritional Biochemistry*, vol. 12, no. 9, pp. 505–511, 2001.
- [75] R. Flamini and D. Tomasi, "The anthocyanin content in berries of the hybrid grape cultivars Clinton and Isabella," *Vitis*, vol. 39, no. 2, pp. 79–81, 2000.
- [76] E. Hebrero, C. Garcia-Rodriguez, C. Santos-Buelga, and J. C. Rivas-Gonzalo, "Analysis of anthocyanins by high performance liquid chromatography-diode array spectroscopy in a hybrid grape variety (*vitis vinifera* x *vitis berlandieri* 41b)," *American Journal of Enology and Viticulture*, vol. 40, pp. 283–291, 1989.
- [77] S. L. Nixdorf and I. Hermosín-Gutiérrez, "Brazilian red wines made from the hybrid grape cultivar Isabel: phenolic composition and antioxidant capacity," *Analytica Chimica Acta*, vol. 659, no. 1–2, pp. 208–215, 2010.
- [78] F. Mattivi, R. Guzzon, U. Vrhovsek, M. Stefanini, and R. Velasco, "Metabolite profiling of grape: flavonols and anthocyanins," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 20, pp. 7692–7702, 2006.
- [79] R. Di Stefano, "Metodichimici nella caratterizzazione varietale," *Rivista di Viticoltura e di Enologia*, vol. 1, pp. 51–56, 1996.
- [80] Q. Tian, M. M. Giusti, G. D. Stoner, and S. J. Schwartz, "Screening for anthocyanins using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry with precursor-ion analysis, product-ion analysis, common-neutral-loss analysis, and selected reaction monitoring," *Journal of Chromatography A*, vol. 1091, no. 1–2, pp. 72–82, 2005.
- [81] I. J. Košir, B. Lapornik, S. Andrenšek, A. G. Wondra, U. Vrhovšek, and J. Kidrič, "Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance," *Analytica Chimica Acta*, vol. 513, no. 1, pp. 277–282, 2004.
- [82] M. Monagas, V. Núñez, B. Bartolomé, and C. Gómez-Cordovés, "Anthocyanin-derived pigments in Graciano, Tempranillo, and Cabernet Sauvignon wines produced in Spain," *American Journal of Enology and Viticulture*, vol. 54, no. 3, pp. 163–169, 2003.
- [83] P. Kebarle and U. H. Verkcerk, "Electrospray: from Ions in solution to Ions in the gas phase, what we know now," *Mass Spectrometry Reviews*, vol. 28, no. 6, pp. 898–917, 2009.
- [84] S. Crotti, R. Seraglia, and P. Traldi, "Some thoughts on electrospray ionization mechanisms," *European Journal of Mass Spectrometry*, vol. 17, no. 2, pp. 85–100, 2011.
- [85] P. Sarni-Manchado, H. Fulcrand, J. M. Souquet, V. Cheynier, and M. Moutounet, "Stability and color of unreported wine anthocyanin-derived pigments," *Journal of Food Science*, vol. 61, no. 5, pp. 938–941, 1996.
- [86] M. Karas, U. Bahr, and U. Gießmann, "Matrix-assisted laser desorption ionization mass spectrometry," *Mass Spectrometry Reviews*, vol. 10, no. 5, pp. 335–357, 1991.
- [87] A. E. Ashcroft, "Protein and peptide identification: the rôle of mass spectrometry in proteomics," *Natural Product Reports*, vol. 20, no. 2, pp. 202–215, 2003.
- [88] Y. Yang and M. Chien, "Characterization of grape procyanidins using high-performance liquid chromatography/mass spectrometry and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 9, pp. 3990–3996, 2000.

- [89] C. G. Krueger, N. C. Dopke, P. M. Treichel, J. Folts, and J. D. Reed, "Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of polygalloyl polyflavan-3-ols in grape seed extract," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 5, pp. 1663–1667, 2000.
- [90] N. Vivas, M. F. Nonier, and N. Vivas De Gaulejac, "Structural characterization and analytical differentiation of grape seeds, skins, steams and Quebracho tannins," *Bulletin de L'OIV*, vol. 77, no. 883-884, pp. 643–659, 2004.
- [91] N. Vivas, M. F. Nonier, N. Vivas De Gaulejac, C. Absalon, A. Bertrand, and M. Mirabel, "Differentiation of proanthocyanidin tannins from seeds, skins and stems of grapes (*Vitis vinifera*) and heartwood of Quebracho (*Schinopsis balansae*) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and thioacidolysis/liquid chromatography/electrospray ionization mass spectrometry," *Analytica Chimica Acta*, vol. 513, no. 1, pp. 247–256, 2004.
- [92] H. A. Weber, A. E. Hodges, J. R. Guthrie et al., "Comparison of proanthocyanidins in commercial antioxidants: grape seed and pine bark extracts," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 1, pp. 148–156, 2007.
- [93] M. F. Nonier, C. Absalon, N. Vivas, and N. Vivas De Gaulejac, "Application of off-line size-exclusion chromatographic fractionation-matrix assisted laser desorption ionization time of flight mass spectrometry for proanthocyanidin characterization," *Journal of Chromatography A*, vol. 1033, no. 2, pp. 291–297, 2004.
- [94] L. Mattoli, F. Cangi, A. Maidecchi et al., "Metabolomic fingerprinting of plant extracts," *Journal of Mass Spectrometry*, vol. 41, no. 12, pp. 1534–1545, 2006.
- [95] P. Arapitsas, M. Scholz, U. Vrhovsek et al., "A metabolomic approach to the study of wine micro-oxygenation," *PLoS ONE*, vol. 7, no. 5, Article ID e37783, 2012.
- [96] A. Cuadros-Inostroza, P. Giavalisco, J. Hummel, A. Eckardt, L. Willmitzer, and H. Peña-Cortés, "Discrimination of wine attributes by metabolome analysis," *Analytical Chemistry*, vol. 82, no. 9, pp. 3573–3580, 2010.
- [97] L. Vaclavik, O. Lacina, J. Hajslova, and J. Zweigenbaum, "The use of high performance liquid chromatography-quadrupole time-of-flight mass spectrometry coupled to advanced data mining and chemometric tools for discrimination and classification of red wines according to their variety," *Analytica Chimica Acta*, vol. 685, no. 1, pp. 45–51, 2011.
- [98] A. Carpentieri, G. Marino, and A. Amoresano, "Rapid fingerprinting of red wines by MALDI mass spectrometry," *Analytical and Bioanalytical Chemistry*, vol. 389, no. 3, pp. 969–982, 2007.
- [99] H. Fulcrand, C. Mané, S. Preys et al., "Direct mass spectrometry approaches to characterize polyphenol composition of complex samples," *Phytochemistry*, vol. 69, no. 18, pp. 3131–3138, 2008.
- [100] G. Mazerolles, S. Preys, C. Bouchut et al., "Combination of several mass spectrometry ionization modes: a multiblock analysis for a rapid characterization of the red wine polyphenolic composition," *Analytica Chimica Acta*, vol. 678, no. 2, pp. 195–202, 2010.
- [101] M. Krauss, H. Singer, and J. Hollender, "LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns," *Analytical and Bioanalytical Chemistry*, vol. 397, no. 3, pp. 943–951, 2010.
- [102] M. De Rosso, A. Panighel, R. Carraro et al., "Chemical characterization and enological potential of Raboso varieties by study of secondary grape metabolites," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 21, pp. 11364–11371, 2010.
- [103] R. Flamini, A. Dalla Vedova, and A. Calò, "Studio sui contenuti monoterpenici di 23 accessioni di uve Moscato: correlazione tra profilo aromatico e varietà," *Rivista Di Viticoltura E Di Enologia*, vol. 54, no. 2-3, pp. 35–49, 2001.