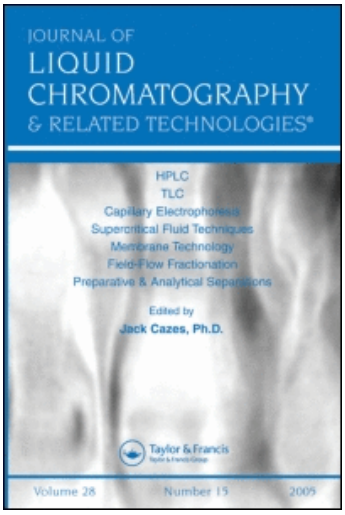

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Stilbenes in Wine by HPLC: Recent Approaches

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To cite this Article Gocan, Simion(2009) 'Analysis of Stilbenes in Wine by HPLC: Recent Approaches', Journal of Liquid Chromatography & Related Technologies, 32: 11, 1598 — 1643
To link to this Article: DOI: 10.1080/10826070902956352
URL: http://dx.doi.org/10.1080/10826070902956352

Journal of Liquid Chromatography & Related Technologies®, 32: 1598–1643, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902956352

Analysis of Stilbenes in Wine by HPLC: Recent Approaches

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Abstract: This review is focused on an HPLC method and its performance in the wine analysis of the stilbenes. All preparation techniques are used in the analysis by liquid chromatography as: direct injection, solid liquid extraction, automated solid phase extraction, and liquid liquid extraction. The HPLC technique with UV, diode array, fluorescence, electrochemical, or mass spectrometry detection systems, has an important place in the field of wine analysis. Also, limit of detection and limit of determination are presented for these types of detectors. The levels of resveratrol in wine, varies from region to region and from one year to another. No region can be said to produce wines with significantly higher levels of *trans*-resveratrol than all other regions, and levels of *cis*-reveratrol follow the same trend as *trans*-resveratrol. Red wine contains a higher quantity of resveratrol than white wine. The average level of *trans*-resveratrol-glicoside (*trans*-piceid) in red wine may be of three times that more than of *trans*-resveratrol.

Keywords: Content of resveratrol in wines, HPLC with several detector types, Identification of resveratrol and their related compounds, Limit of detection and quantification, Sample preparation, Stilbenes, Wines

INTRODUCTION

The polyphenols are the biggest classes containing natural products: phenolic acids and derivatives, flavonoids, lignans, and stilbenes compounds. The flavonoid compounds have been characterized and classified

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according to chemical structure. They are usually subdivided into five subgroups: flavonols, flavones, flavanones, flavan-3-ols, and anthocyanidins. The most representative compound for the stilbenes class is the resveratrol and their related compounds.

In 1976, Langcake and Pryce[1] demonstrated that resveratrol was produced by "*Vitis vinifera* as a response to infection or injury." This compound occurs naturally in grapes and a variety of medicinal plants, where it functions as a phytoalexin that protects against bacterial and fungal infections and other stress factors.[2] It was demonstrated to provide resistance in grapes to fungal disease.[3] It is synthesized almost exclusively in the skins of *Vitis vinifera* grapes, but is also synthesized in the seeds of muscadine grapes.[3,4]

The traditional Japanese folk remedy demonstrated that resveratrol was the primary active ingredient in a medicine composed of the dried powdered root of the Japanese knotweed (*Polygonum cuspidatum* Sieb. Et Zucc.).[5]

Data from the United States of America showed that generally the consumption of fatty food increased the rate of coronary heart disease, but data from France did not follow this pattern. This phenomenon has become known as "the French Paradox."[6] In 1992, Renaud and Lorgeril demonstrated that wine consumption was statistically the only factor correlated to the reduction in coronary heart disease.[7,8]

Resveratrol was reported to have numerous health benefits including, i.e., cardiovascular protective and the reduction of the heart diseases,[9–15] anti-cancer and anti-inflammatory proprieties,[16–28] antioxidant activity,[29,30] antibacterial activity,[31] and was found to inhibit herpes simplex virus types 1 and 2 (HSV-1 and HSV-2).[32]

Grapes are probably the most important source of resveratrol for humans, since the compound is also found in one of the end products of grapes, i.e., wine. Resveratrol is found in all variety of wines, but the highest amount is found in red wines.

The present review is focused on recent approaches on separation, detection, and identification of stilbenes from wine by modern methods as high performance liquid chromatography (HPLC) with UV, and diode array (DA), fluorescence (FL), chemiluminescence (CL), electrochemical (EL), and mass spectrometry (MS) detectors (D). The possibility of identification of the resveratrol compounds and their glycosides in wine by using HPLC-DAD and HPLC-MS or HPLC-MS/MS, is discussed. The limits of detection and quantification of several methods are also, presented. Finally, the content of resveratrol in wines produced in different wineries and vintages from several countries and continents is discussed here.

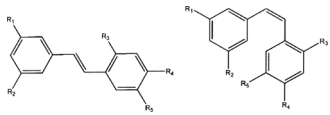
CHEMICAL STRUCTURE OF STILBENES

Resveratrol (3,5,4′-tryhydroxystilbene) is a polyphenolic phytoalexin compound classified as a stilbene. It exists as two geometric isomers: *trans-(E)* and *cis-(Z)* isomeric forms. The *trans*- form can undergo isomerisation to the *cis*- form when exposed to UV irradiation. Resveratrol also exists in a glucoside *trans* and *cis* form called piceid (5,4′-dihydroxy-3-glucopyranosyl stilbene).

Structural features of mechanistic importance were described from experimental crystal structure and previous calculations on resveratrol by Caruso et al.[33] The molecular structure of resveratrol shows relative coplanarity of the *trans*-resveratrol skeleton. Moreover, the molecular packing in the solid state reveals an extensive hydrogen bond network.

Caruso et al.[33] described, from experimental crystal structure and previous calculations on resveratrol, the structural features of mechanistic

Table 1. The structures of stilbenes



Compound	R1	R2	R3	R4	R5
Stilbene	H	H	H	H	H
trans-Resveratrol	OH	OH	H	OH	H
trans-Pterostilbene	H_3CO	H_3CO	H	OH	H
trans-Resveratrol-3-*O*-Glu (*trans*-Piceid)	*O*-Glu	OH	H	OH	H
trans-Resveratrol-3-*O*-Glc (*trans*-Piceid)	*O*-Glc	OH	H	OH	H
trans-Astringin	*O*-Glc	OH	H	OH	OH
Astringinin	OH	OH	H	OH	OH
Rhapontigenin	OH	OH	H	OCH_3	OH
Rhaponticin	*O*-Glu	OH	H	OCH_3	OH
Piceatannol	OH	OH	H	OH	OH
Pinosilvin	OH	OH	H	H	H
Pterostilbine	OCH_3	OCH_3	H	OH	H
cis-Stilbenes					
cis-Resveratrol	OH	OH	H	OH	H
cis-Resveratrol-3-*O*-Glu (cis-Piceid)	*O*-Glu	OH	H	OH	H
cis-Resveratrol-3-*O*-Glc (cis-Piceid)	*O*-Glc	OH	H	OH	H

importance. The molecular structure of resveratrol shows relative coplanarity of the *trans*-resveratrol skeleton. Also, the molecular packing in the solid state reveals an extensive hydrogen bond network that elucidates the flip flop of the three hydroxyl groups that alternately form and break H bonds with each of the neighboring phenolic oxygens. The experimental and theoretical results from this study help to explain the biological activity of this simple substituted stilbene.

There are many forms of oxidative dimerization of resveratrol, namely *viniferin* isomers, which are known today. If the pure *trans*-resveratrol is exposed to UV irradiation for periods between 3 and 5 min, partial conversion to *cis*-resveratrol occurred without other modifications of the sample, or destruction of either isomer or the appearance of other products.[34] A 418 μmol/L *trans*-resveratrol solution exposed to 366 nm UV irradiation for 2 h, became 90.6% *cis*-resveratrol. After that, it was exposed to laboratory fluorescent lighting conditions and dropped to 86.1% over 60 days.[35] The structures of some stilbenes and viniferin isomers are presented in Table 1.

SAMPLE PREPARATION

The samples should be immediately stored in the refrigerator at 4°C in a dark glass until analysis. Samples which have been opened must be protected against sunlight and stored at 4°C and analyses will be performed within a few days.

Generally speaking, the sample preparation of wine is not necessarily a very complicated operation. The usual method regarding the sample preparation of wine can be presented as following:

- Direct injection into chromatograph,
- Solid phase extraction,
- Automation of solid phase extraction,
- Liquid-liquid extraction, and
- Extraction from solid matrix by maceration.

The liquid-liquid (L-L) extraction of the sample is performed by partitioning of the different substrates between the aqueous phase and the organic extracting solvent phase is in accord with Nernst's low. Any neutral species will distribute between two immiscible solvents, such that the ratio of the concentrations remain constant, K_D.

The distribution constant K_D is equal with ratio:

$$K_D = \frac{C_o}{C_{aq}} \tag{1}$$

where C_o is the concentration of compound a in organic phase and C_{aq} is the concentration of a in the aqueous phase.

The fraction, E of an analyte a removed from the aqueous sample by L-L extraction is:

$$E = 1 - \frac{1}{(1 - K_D V)^n} \tag{2}$$

where, V_o/V_{aq} is ratio between the organic- and aqueous-phase volumes, and n is the number of extraction.

The wine sample is shaken with an immiscible organic solvent in which the components are soluble. The most common extraction solvents are diethyl ether and ethyl acetate (Table 2). The organic layer is separated, concentrated, and injected into the high performance liquid chromatography system.

Solid-phase extraction (SPE) is an alternative to L–L extraction. As known, the SPE technique has been applied to clean, to fractionate, solvent change over, and compound concentration. Furthermore, with SPE, many of the problems associated with L-L extraction can be prevented, such as incomplete phase separations, less than quantitative recoveries, and large consumption of solvent. SPE is more efficient than L-L extraction, yields a quantitative extraction that is easy to perform, and solvent use and lab time are reduced. The SPE procedure can be incorporated into the HPLC system.

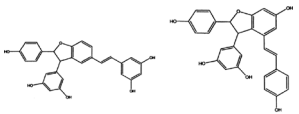
In SPE there is the same separation process as in reversed phase chromatography. The stationary phase is formed of a non-polar sorbent, for example, a *n*-octadecylsilane or a *n*-octylsylane (C_{18} or C_8) bonded-phase on silica.

The retention of a compound is determined by its polarity and experimental conditions: mobile and stationary phase. The nature of the reversed phase retention can be approximated by a partition process: sample molecules will be partitioned between the polar mobile phases (matrix of the wine) and non-polar C_{18} or C_8 stationary phase from a small column namely "cartridge." The more hydrophobic compounds (stilbene derivatives) from the wine are retained more strongly, than other polar compounds. For elution of the wine compounds from cartridge polar solvents are used.

SPE is performed in a five step process:[36]

- Select the proper SPE cartridge,
- Condition the proper SPE cartridge,
- Add the sample,
- Wash the SPE cartridge, and
- Elute the compounds of interest.

Table 2. Sample preparation



Sample	Methods	Comments	Ref.
Spanish red Vitis vinifera wines	None	Direct injection in HPLC system after filtration through Whatman Anapore membrane filters (Anodisc, 0.2 μm); two replicates for each sample.	37
Twenty-six varietal Spanish white wines	None	The samples were injected by a Rheodyne injection valve with a 100 mL fixed loop into HPLC system.	38
Spanish red wines	None	Direct HPLC injection, after filtration through 0.2 μm membrane filters.	39
		For hydrolysis digestion with β-glucosidase 1 mL of wine was neutralized with NaOH to pH 6.0, then 4 mg of β-glucosidase was added, and digestion for 18 h at 25°C under darkness.	40
Red wines from different Spanish regions	None	The samples were filtered through a 0.45 μm membrane and direct injection into HPLC system or colorimetric determination of polyphenols content.	41
Comercial wines (Portugal, France)	None	Direct injection into HPLC system.	42
Red and white wines, Bohemian and Moravian vineyard regions	None	Direct injection into HPLC system.	43

(*Continued*)

Table 2. Continued

Sample	Methods	Comments	Ref.
Aragon red and rose wines	None	Direct injection into HPLC system.	44
French red wines	None	Direct injection into HPLC after filtration.	45
Wines produced in Greece	None	Direct injection into HPLC system.	46
Greek red and with wines	None	Injection was made by means of a Rheodyn injection valve with 20 μL, fixed loop into HPLC system.	47
Brazilian commercial red wines	None	Direct injection into HPLC system Merlot wine sample diluted of six times.	48
Hungarian Villany red wines	None	Direct injection into HPLC system.	49
Hungarian Villany wines	None	Direct injection with Reodine (20 μL loop) into HPLC system.	50
Red and white wines (Polgar Winery and Bock Winery, Villany, Hungary)	None	Direct injection into HPLC system.	51
Hungarian wine (Tokaj aszu)	None	Direct injection into HPLC system.	52
Turkey wine	None	Wine and must samples of 0.5 mL were centrifuged (10,000 g for 5 min) and were pleased in a thermostat auto sampler (+4°C), and 100 mL was injected into HPLC system.	53
AOC Bergerac France	None	Wine samples were filtered and 100 μL was directly injected into HPLC system.	54
Wines Viti-RD Company in Villetelle Herault, France	None	Wines sample were filtered through a 0.2 μm cellulose regenerated filter and then injected.	55
Italian wines	None	Direct injection into HPLC-UV, HPLC-FL and HPLC-MS system. All the samples were filtered through a 45 μm membrane.	56 57
Brazilian wines	None	A 25 μL amount of wines was injected into HPLC system after filtration.	58

Commercial Chinese red wines	None	Wine samples were filtrated. The filtrated solutions were injected directly into HPLC with DA detection. A 0.05 mL aliquot of the filtrated wine samples was diluted to 5.0 mL with mobile phase and injected directly into HPLC with CL detection.	59
Commercial Romanian red and white wines	None	Direct injection into HPLC after diluted 1:10 with HPLC water and filtrated.	60
Sicilian red wines	None	Samples were filtered through a 0.45 μm membrane filter (Millipore) and direct injected into HPLC system.	61
Selected wines	None	Direct injection into HPLC with EC detection.	62
Commercial wines and musts	None	Samples were filtered through a 0.2 μm; nylon Millipore chromatographic filter, diluted when was necessary and injected.	63
Wine	None	Wine and resveratrol samples were filtrated through 4 mm PTFE 0.45 μm; and analysis by HPLC.	64
Wines	None	Untreated wine (20 μL) was directly injected into HPLC, and 1 μL sample of HPLC eluates or hydrolyzed fractions was injected into a GC-MS system.	65
Wines	None	Direct injected into HPLC system.	66 67
Gewurtztraminer and Muller-Thurgau wine extracts	SPE	Volumes of 5 mL were diluted to 15 mL with water and solution was passed through a Sep-PakC_{18} cartridge previously activated by passage of 3 mL methanol followed by 5 mL water. After the sample cartridge was loaded, the cartridge was washed with 10 mL water. Resveratrol was recovered with 5 mL methanol, and was reduced to 1.5 mL by Rotavapor before analysis.	68

(*Continued*)

Table 2. Continued

Sample	Methods	Comments	Ref.
Wine	SPE on C_8	1 mL was diluted 1:1 with water and extracted on a preconditioned C_8 cartridge and eluting with ethyl acetate. The phenolic compounds were derivatised with TMS/pyridine, and analyzed by GC-MS.	69
Italian wines	Sep-Pak C_{18}	In order to identify the compound suspected to be astringin, a concentrated wine sample was needed. A Sep-Pak C_{18} column was preconditioned with 3 mL of methanol and washed with 6 mL of aqueous 1.8 mM HCOOH. The wine sample (1 mL) was loaded on column. After than was washed with 3 mL of distilled water. The sample was eluted by 2 mL of methanol and directly collected in a vial. The eluate was evaporated and residue was dissolved in 100 μL of IS solution in methanol (1 ng/μL and aliquots of 5 μL solution were analyzed by LC/TOFMS.	56
Italian wines	SPE on C_{18}	10 mL of red wine and 20 mL of white wine were loaded on a preconditioned C_{18} column. The column was conditioned with 3 mL EtOAc, followed by 3 mL of EtOH 96% and 3 mL EtOH 10% (twice). Wine samples were adsorbed by gravity, and then the column was dried under nitrogen flux for 45 min. Tyrosol and hydroxytyrosol were eluted with 3 mL of EtOAc, and 1 mL was collected for analysis. 50 μL of wine extract was treated with 50 μL of BSTFA, and then held at 60°C at room temperature overnight, and analyzed by GC-MS.	70
Ontario wines	Diluted and SPE	SPE followed derivatization GC-MS	71

Red wine	SPE on C_{18}	Conditioned with 10 mL ethyl acetate, 10 mL methanol, and 10 mL water. The sample, 10 mL of red win, was eluted from cartridges with 10 mL of ethyl acetate. The residue was dissolved in 0.5 mL of methanol and injected in the CE system.	72
Canary Islands (Spain) wines	SPE on Sep-Pak Plus C_{18}	C_{18} cartridge was previously conditioned with 4 mL of methanol, followed by 4 mL of water and dried with nitrogen gas stream. The 5 mL of wine were introduced and the compounds were eluted with 3 mL of methanol. The solution was filtering and injected into a HPLC system.	73
Greek red wine	SPE on C_{18} Sep-Pak	The cartridges were condition by washing with 3 mL of ethyl acetate, followed by 3 mL ethanol (96%) and 5 mL of ethanol (12%) twice. After passing the sample of 2 mL wine through the cartridge was washing with 10 mL water followed by 10 mL ethanol (12%) adjusted to pH 8.0. After dried 15 min by a constant flow of nitrogen. For eluted resveratrol was used 10 mL ethyl acetate and concentrated and redissolved in 1 mL methanol and injected into a HPLC system. The mean recovery of *trans*-resveratrol over the range 0.6–1.2 mg/L was 99.4%, but at the conc. range 2.4–3.6 mg/L was 100.9%, and for 6 mg/L was 92%.	74
Palomino fino grapes	SPE	The juice sample was performed using C_{18} LiChrospher cartridges. Prior to extraction stage the sample was centrifuged and filtered and injected into HPLC system.	75
	Extraction	Sample of skin were macerated with a mixture methanol/HCl at 0.1% (25 g/50 mL) for 30 min ultrasound. After the	

(*Continued*)

Table 2. Continued

Sample	Methods	Comments	Ref.
		extraction, the sample was centrifuged and filtered prior to direct injection.	
Wine	Tandem SPE LC/MS	To 1 mL of wine was added 1 mL of the IS solution (trihydroxyflavanone, 0.68 mg/L). After mixing was centrifuged and clear solution was applied on SPE columns, which were tandem, consisted with Sep-Pak Plus C_{18} and PS-1 columns. These have been preconditioned successively with methanol (5 mL) and ammonium acetate (20 mM, pH 5.5, 5 mL). The column was washed with 2.5 mL of methanol/20 mM ammonium acetate, pH 5.5 (10:90, v/v) and eluted with 1 mL of methanol/20 mM ammonium acetate, pH 5.5 (85:15, v/v), and 10 μL of eluate was injected into LC/MS system.	76
A variety of different types of wines from Spain	Automated SPE	Sample was filtered through nylon filters of 0.45 μm before subsequently undergoing an SPE, for the purpose of cleaning and pre-concentrating them before injection into the HPLC-DAD-MS system. The cartridges used were LiChrolut EN, first was conditioned with 5 mL of methanol and followed by 3 mL of water. A sample of 5 ml of wine was added internal standard and was diluted 1:1 with water. The SPE stage was performed by a totally completely automated method using a semi-flexible and automatic robotic system. All the steps and flow rate were previously optimized.	77

Wine	SPE workstation	The cartridge was subsequently wash with 3 mL water, 2 mL methanol, precondition with 2 mL water, load sample 2.1 mL, wash with 2 mL water, and 1 mL of 15% methanol, (is optimal wash solvent for eliminating possible polar interference) and resveratrol was completely recovery when 1 mL methanol was used as the elution solvent.	78
Wines Alentejo region Portugal	Liquid-liquid (L-L) SPE None	L-L extraction was performed with *n*-hexane before the injection. Extraction using C_{18} before injection. Diluted in a 12% alcoholic aqueous solution, filtered and injection in HPLC system.	79
Red wines Navarra, Spain	Liquid-liquid	A volume of 50 mL of wine was concentred to 15 mL, under vacuum at 30°C and extracted three times with diethyl ether (15 mL) and the same with ethyl acetate.	80
Wines Canary Island	Liquid-liquid	Wine samples were adjusted to pH 2 by adding 0.1 M hydrochloric acid. Then, 5 mL of wines were extracted twice with 5 mL of diethyl ether for 20 min using a Selecta Rotabit at 180 rpm. Organic layer was separated and evaporated in nitrogen gas stream. The dry residue was dissolved in methanol-water (1:1, v/v), filtered and injected into HPLC system.	81
Red wines China	Liquid-liquid	Extractions of wine polyphenols were performed to the method [83] with the following modifications: 100 mL deionized water was added to 100 mL wine and mixture was extracted with 80 mL ethyl acetate. The organic phase was concentrated under 30°C and residue was dissolved in 5.0 mL methanol-water (1:1, v/v).	82 83

(*Continued*)

Table 2. Continued

Sample	Methods	Comments	Ref.
Red wine Southwestern France	Liquid-liquid	Polyphenols from the ethyl acetate extracts of red wine were fractioned using a four-step process: solvent extraction, ion-exchange column chromatogr., centrifugal partition chromatogr., and semi preparative HPLC, which resulted in the isolation of 22 compounds.	84
Grapes, rachises, leaves from Moravian vineyard regions	Extraction	The fresh plant material was grinding and drying. The extraction was performing with 80% ethanol in darkness at 20°C and lasted 24 h. The liquid was separated by filtration and it is ready for injection.	43
Vitis vinifera leaves	Extraction	Three pieces of Chasselas leaves were cut in three different *Plasmopara viticola* sporulating zones or three pieces from Chasselas irradiated leaves and separately crushed into 300 μL of methanol placed into a glass tube and then centrifuged at 14000 rpm. Aliquots (10–30 μL) of the supernatant were directly injected.	85

IS – internal standard; EtOAc – ethyl acetate; EtOH – ethyl alcohol; BSTFA – bis(trimethylsilyl)trifluoroacetamide; PTFE – poly(tetrafluoroethylene) TOF – time-of-flight.

A lot of experimental condition for SPE from wine are summarized and presented in Table 2.

HPLC METHODS

Separation and Detection Methods

The separation by HPLC removes the need for derivatization of stilbenes and has been used extensively with UV and/or fluorescence detection.

The resveratrol is most the commonly analyte identified and quantified in a very large variety of wines by HPLC analysis. Reversed phase high performance liquid chromatography (RP-HPLC) is typically more convenient and robust than other forms of liquid chromatography, such as normal phase (NP-HPLC).

The column packings with *n*-octadecylsilane (ODS or C_{18}) bonded phase are used for separations of the resveratrol and their related compounds. The number of studies that used a polar bonded phase, NP-HPLC of cyano (CN) bonded phase on a silica support is less than RP-HPLC, as can seen from Table 3. Furthermore, gradient liquid chromatography is more frequently used than isocratic elution. The mobile phase used with RP-HPLC columns is a mixture of acetonitrile and/or methanol in combination with water containing small amounts of acid. Details about the eluent systems, HPLC columns, and detection methods are summarized in Table 3.

In some cases, elevated column temperature above ambient, was used. Currently, there are no studies in the literature that compare the effect of column temperature on analysis of resveratrol by liquid chromatography.

Detection and quantification of resveratrol and their related compounds were performed by UV absorption at 306–308 nm wavelengths and diode-array detection (DAD), because they allow simultaneous detection of multiple wavelengths. Other less common methods used for detection are the following: fluorometric detection (FLD), electrochemical detection (ECD), and mass spectrometry detection (MSD), (Table 3).

Generally speaking, the HPLC method is preferable for the determination of the polyphenolic compounds from samples of red wine. The analyses were performed in a HPLC Waters system (Milford, MA), USA) equipped with two pumps (Models 510), an automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20 μL loop), a programmable FLD (Model 470) in series with a tunable absorbance detector (Model 486).[81] The chromatographic conditions are presented

Table 3. HPLC analysis of reveratrol and other related compounds from wines

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
Isocratic elution				
trans-resveratrol	C_{18} Lichrokart (250 × 4.6 mm, 5 μm particles)	Water/acetonitril/acetic acid (70/29.9/0.1, v/v); 1.0 mL/min;	UV 310 nm	79
trans-resveratrol	Octadecyl (250 × 4.6 mm, 5 μm) 24°C	Water/acetonitrile (75/25, v/v) pH 3 using H_3PO_4 1.5 mL/min	UV-vis 190–360 nm	48
cis-and *trans*-polydatin (resveratrol-3-*β*-glucoside)	LiChrospher 100 CN (250 × 4. mm, 5 μm)	Water/acetonitrile/methanol (90:5:5, v/v) 1 mL/min	UV 306 nm	66
trans- and *cis*-resveratrol *trans*- and *cis*-resveratrol glucoside	LiChrospher 100 CN (250 × 4. mm, 5 μm)	Water/acetonitrile/methanol (90:5:5, v/v) 1 mL/min	UV 306 nm DAD	65
trans-resveratrol	Nucleosil C_{18} (150 × 4.6 mm, 5 μm) Benzyldimethylsilyl bonded phase (150 × 4.6 mm, 7 ± 2 μm)	0.05 mol/L $NH_4H_2PO_4$ in 25% (v/v) aqueous acetonitrile	UV 200–800 nm ECD with glassy carbon electrode and silver/silver chloride reference electrode	62
trans- and *cis*-resveratrol and quercetin	Luna C_{18} (2) (250 × 2 mm, 5 μm) 30°C	1% (v/v) formic acid aqueous solution/acetonitrile/2-propanol (70:22:8, v/v); 0.2 mL/min	UV/DAD, MS 306 nm ESI *trans*- resveratrol m/z 227	61

trans-resveratrol	Capcellpak C_{18}UG120 (250 × 1.5 mm, 5 μm)	Methanol/20 mM ammonium acetate pH 5.5 (55:45,v/v); Flow rate 0.15 mL/min.	LC-MS, ESI $[M\text{-}H]^-$ m/z 227	76
trans- and *cis*-resveratrol	ODS (100 × 2. mm, 3 μm)	20 mM sodium acetate, 0.5 mM EDTA pH 4.5 and 18% of acetonitrile 0.4 mL/min	Multi-chanal ECD +700 mV	78
trans-resveratrol	Zorbax Eclipse XDB-C_8 (150 × 4.6 mm, 5 μm) 25°C	Methanol/water (35:65, v/v) 1.0 mL/min	DAD 306 nm and CLD	59
trans- and *cis*-resveratrol	120–5-C_{18} Nucleosil (250 × 4.0 mm, 5 μm)	25% acetonitrile, 0.1% H_3PO_4 and NaCl (c = 5 mM/L) in water. 1.0 mL/min	UV Maximum 306 to 320 nm, and 295 respectively ECD at 0.75 V	43
trans- and *cis*-resveratrol	Zorbax SB-C_{18} (100 × 3.0 mm 3.5 μm)	Ammonium acetate 1 mM/acetonitrile (73:27, v/v) 1 mL/min	MS/MS with APCI negative m/z 227	60
Gradient elution				
trans- and *cis*-reveratrol	Nova-Pak C_{18} (150 × 3.9 mm, 4 μm)	A. Methanol/acetic acid/water (10: 2: 88,v/v) B. Methanol/acetic acid/water (90: 2: 8, v/v); Solvent A. from 100% to 85%, 0–15 min, 85% to 50%, 15–25 min, 50% to 30%, 25–34 min; 1.0 mL/min.	Absorbance $\lambda = 280$ nm; FLD $\lambda_{ex} = 360$ nm $\lambda_{em} = 374$ nm	73
trans- resveratrol, chatechin, epichatechin	C_{18}Hypersil H5 ODS (250 × 4.6 mm)	A. 9% acetonitrile, 91% (5% aqueous acetic acid) B. 25% acetonitrile, 75% (5%	FLD 11 min $\lambda_{ex} = 280$ nm $\lambda_{em} = 315$ nm and for chatechin, and epichatechin	53

(*Continued*)

Table 3. Continued

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
		aqueous acetic acid) C. 70% acetonitrile, 30% (5% aqueous acetic acid) Step gradient: 100% A, 0–10 min; 100% B, 10–11 min and held 11–22 min. To prepare the column for the next run 5 min 100% C and then 100% A for 15 min, 1 mL/min	$\lambda_{ex} = 324$ nm $\lambda_{em} = 370$ nm until 20 min	
trans- and *cis*-reveratrol *trans*- and *cis*-piceid *trans*-astringin and (+)-catechin, (−)-epicatechin and pallidol astilbin and dihydromyricetin-3-*O*- rhamnoside	Prontosil C_{18} (250 × 4.0 mm, 4 μm)	A. Water/trifluoro acetic acid (99:1, v/v); B. acetonitrile/solven A (80:20, v/v) for stilbenes 15% B, 0–10 min; 15–18% B, 10–13 min; 18% B, 13–15 min; 18–23% B, 15–17 min, 23–25% B, 17–21 min, 25% B, 21–24 min, 25–32% B, 24–28 min, 32% B, 28–30 min, 32–40% B, 30–33 min, 40% B, 33–38 min 40–70% B, 38–44 min 70–80% B, 44–47 min,	FLD step: nm 280/310, 290/390, 260/400, 300/390, 260/400	54

		80–90% B, 47–52 min, 90–100% B, 52–53 min, 100% B, 53–58 min, 10 min equilibrium; GE start 18% B and finished 100% B in 40 min. 1.0 mL/min		
Stilbene: *trans*-reveratrol *trans*-piceatannol *trans*-piceid *trans*-astringin	Kromasil 5 μm C_{18}	A. methanol/1.8 mM formic acid, and B. water/1.8 mM formic acid. Zero-time were 70% B and 30% A; a linear gradient to 40% B in 30 min. After then changed to 0% B in 2 min and maintained for 3 min in order to wash the column. 150 μL/min	MS, MS/MS TOF-MS ESI m/z $[M\text{-}H]^-$ t_R, min 227.0; 25,4 243.0 19.7 389.2 17.1 405.0 12,1	56
trans-reveratrol	Kromasil 5 μm C_{18}	A. methanol/formic acid 0.09% and B. water/formic acid 0.09%. Zero-time 70% B and 30% A; a linear gradient to 30% B in 40 min. After then changed to 0% B in 5 min and maintained for 5 min to wash the column. 150 μL/min	HPLC-UV, HPLC-FL, HPLC-MS	57
trans-resveratrol *trans*-piceid	C_{18} (250 × 4.6 mm, 6 μm)	A. methanol/water/acetic acid (10:90:1, v/v); B. methanol/water/acetic acid	UV DAD UV-vis and MS, APCI negative ion	50 51

(*Continued*)

Table 3. Continued

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
		(90:10: 1, v/v); B: 0–40%, 0.0–18.0 min 40–100%, 18.0–25.0 min 100%, 25.0–27.0 min 1 mL/min		
Resveratrol-3-*O*-β-D-glucoside and (36 compounds) flavonoid-glycosides or carbohydrate derivatives of phenolic acids,	LiChropher 100-RP-18 (250 × 4.6 mm, 5 μm) 20–25°C	A. water/formic acid (92:2, v/v); B. acetonitrile/water/formic acid (80:18:2, v/v); Isocratic: 100% A 5 min, GE: 10% to 30% B in 25 min and 30% to 100% in 5 min; and isocratic 100% B 5 min, re-equilibrating the column. 0.8 mL/min	UV-vis 280–310 and 520 nm ESI- and SSI-MS	52
trans-resveratrol and thirteen polyphenols, and anthocyanins	C_{18} (250 × 4.6 mm, 6 μm)	A. methanol/water/acetic acid (10:90:1, v/v); B. methanol/water/acetic acid (90:10: 1, v/v); 0–40% B, 0–18 min 40–100% B, 18–25 min 100%, 25–27 min 1.5 mL/min	UV 306 nm	49
trans-resveratrol	Spher ODS C_{18} (250 × 4. mm, 5 μm) 40°C	A. water/acetonitrile (7:3,v/v), B. water/methanol (5:5, v/v) Solvent A from 0 to 18 min,	DAD UV-vis 208 nm 308 nm	46

		then from. A 100 to B 100% in 1 min, and B 100 t0 A 100% in 6 min. 0.6 mL/min.		
trans-resveratrol	ODS Hypersil (250 × 4. mm, 5 μm) 40°C	A. acetonitrile B. water containing perchloric acid (0.6 mL/L) A 5% 0 min A 5 to 50%, 0–5 min 50–60%, 5–15 min 60% 15–30 min to wash the column return the initial conditions for 5 min 1 mL/min	DAD 310 nm	74
trans- and *cis*-resveratrol	ODS C_{18} (250 × 4. mm, 5 μm) 40°C	A. water/methanol (5:5, v/v) B. water/acetonitrile (7:3, v/v) Elution solvent A from 0 to 18 min then from A 100% to B 100% in 1 min, and from B 100% to A 100% in 6 min to re-estabilish the initial conditions. 0.6 mL/min	DAD 285 nm 306 nm	47
trans- and cis-resveratrol	Nucleosil, C_{18} 120 (250 × 4.0 mm, 5 μm) 40°C	A. acetic acid 12% in water, pH 2.4 B. acetic acid 12% pH 2.4/acetonitrile (20:80, v/v) B 18% 0 min 18–18% 0–11 min 18 – 23% 11–18 min 23–100%, 18–31 min 1,5 mL/min	UV 285 nm and 306 nm	44

(*Continued*)

Table 3. Continued

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
trans- and cis-resveratrol and *trans- and cis*-resveratrol glucoside	Symetry C_{18} (150 × 2.1 mm, 5 μm)	A. adjusted water, pH 2,5 with H_2SO_4; B. acetonitrile A%/B%, min 100/0, 0: 50/50, 60; 50/50, 90; 0/100, 100; 100/0, 110; 0.2 mL/min	UV 280 nm and 305 nm MS SIM mode, at m/z 228	77
trans-resveratrol *trans*-piceid *cis*-piceid	LiChropher 100-RP-18 (250 × 4 mm, 5 μm) 35°C	A. water/0.05% trifluoroacetic acid (TFA) B. methanol/acetonitrile/ (60:40) + 0.05% TFA 0–10% B, 0–5 min 10–15% B, 5–40 min 15–35% B, 40–55 min 35–10% B 20 min for equilibration column 1 mL/min	DAD 306 nm	39
cis- and *trans*-resveratrol and piceid isomers	Nucleosil, C_{18} 120 (250 × 4.0 mm, 5 μm) 40°C	A. Acetic acid aqua sol., pH 2.4, B. 20% phase A whit 80% acetonitrile, 82% A, 0–10 min, 82–77% A, 10–17 min, 77–75,5% A, 17–21 min 75,5–68,5% A, 21–27 min 68,5–0% A, 27–30 min 1.5 mL/min	DAD UV-vis 285 nm, 306 nm	37

cis- and *trans*-resveratrol and piceid isomers	Chromolite Performane RP-18e (100 × 4.6 mm)	A. water/acetic acid (94:6, v/v), B. water/acetonitrile/acetic acid (65:30:5, v/v) 85% 0 min 85% A-70% 0–10 min 70% A-20% 10–17 min 20% A-0% 17–18 min 0% A-85% 18–20 min 4.0, 7.0, 7.0, 7.0, 4.0 mL/min	UV-vis 285 and 306 nm	55
trans-resveratrol gallic acid quercitin rutin	ODS Hypersil (250 × 4.0 mm, 5 μm)	A. acid acetic, B. methanol, C. water A/B/C, (v/v), time min 5:15:80, 0–5, 0.4 mL/min 5:20:75, 5–30, 0.5 mL/min 5:45:50 30–50 min 0.5 mL/min	UV 306 nm, 272 nm, 373,256 nm 360,259 nm	41
Cis-and *trans*-polydatin (resveratrol- 3-*β*-glucoside)	ODS Hypersil (250 × 4.0 mm, 5 μm)	Acetic acid (pomp A) Methanol (pomp B) Water (pomp C) Zero-time were 5% A, 15% B, 80% C at 0.4 mL/min. After 5 min 5% A, 20% B, 75% C at 0.5 mL/min and at 30 min to 5% A, 45% B, 50% C until 40 min. After then 10 min equilibrium period with the zero-time solvent mixture.	DAD 306 nm	66

(*Continued*)

Table 3. Continued

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
trans-resveratrol and pterostilbene	C_{18}	GE. Metanol/acid formic (50 mM)	UV and FLD	67
trans- and *cis*-reveratrol, *trans*- and *cis* polydatin chatechin, epichatechin, rutin, quercetin	ODS Hypersil (250 × 4 mm, 5 μm)	Acetic aci/methanol/H_2O %A/B/C; min; mL/min 5/15/80; 0; 0.4 5/20/75; 5; 0.5, 5/45/50; 30; 0.5, 5/45/50; 40; 0.5, 5/15/80; 50; 0.4	DAD 265, 280, 306 nm, 317, and 369	92
trans- and *cis* resveratrol, standard	HP ODS Hypersil (100 × 2.1 mm, 5 μm) 40°C	Water/methanol %A/B, min 100/0, 0; 0/100, 15; 100/0, 16; 100/0, 21 0.5 mL/min	DAD 306, 286 nm	64
chatechin, epichatechin, rutin, *trans*- and *cis* resveratrol and quercetin	Spherisorb ODS-2 (150 × 4 mm, 5 μm) room temperature	Acetonitril/5% aqueous acetic acid, %A/B, min 9/91, 0; 9/91, 10; 25/75, 11; 25/75, 22; 70/30, 23; 70/30 28; 9/91, 29; 9,91 44 1 mL/min	DAD 306 nm Fluorescence t_R(min) 4.1, 8,5, 14,4 17,2, 19.5, 25.0	63
trans- and *cis* resveratrol; *trans*-, *cis-piceid; trans-ε-, cis-ε*-viniferin; *trans-δ-, cis-δ*-viniferin; *trans-, cis*-pterostilbene	C_{18} Lichrospher (250 × 4,6 mm, 5 μm) 25°C	Acetonitrile/water: 1 min isocratic (20:80%); liner gradient, 20/80%–75/25% 30 min; 75/25%–100%, 2 min; 100% 3 min; 20/80%, 1 min; isocratic 20/80% 4 min; 1 mL/min	*trans* ε- and *trans* δ-viniferin UV 307 nm MSD trap in ESI mode MS^2	85

trans-astringin, *trans*-piceid, *trans*- and *cis* resveratrol, ε-viniferin, δ-viniferin	Nucleosil C_{18} (250 × 4 mm, 5 μm) 30°C	A. acetic acid in water, pH 2.4; B. 20% phase with 80% acetonitrile; with GE 0,5 ml/min	UV 280, 286, 306 and 321 nm t_R (min) 34.51, 40.23 48.96, 49.33, 50.39, 52.22	42 58
trans-resveratrol and sixteen phenolic compounds	Nova-Pak C_{18} (150 × 3.9 mm, 4 μm)	A. metanolic/acetic acid/water (10:2:88, v/v); B. methanol/acetic acid/water (90:2:8, v/v); A%/B%: 100/0 0 min; 85/15, 15 min; 50/50, 25 min; 30/70, 34 min 1.0 mL/min	UV 280 nm FLD λ_{ex} 278 nm λ_{em} 360 nm over 17.5 min and λ_{ex} 330 nm λ_{em} 374 nm over 16.5 min	81
Standard: *trans*- *cis*-resveratrol, *trans*- and *cis-piceid,* seven HBA, five HCA, seven flavan-3-ols, seven flavonols, four flavanones,	Zorbax SB-C_{18} (250 × 4.6 mm, 5 μm) 25°C	A. 1% acetic acid in water; B. 1% acetic acid in methanol; 10–22%B, 0–25 min; 22–50%B 25–45 min; 50–95%B 45–55 min 95%B 55–60 isocratic 95–10%B 60–63 min 10% isocratic 63–66 min 1. mL/min	LC/UV-ESI- MS/MS t_R (min) 44.4; 48.7 37.45; 45.5 $[M-H]^-$ 227(185, 159) 227(185, 159) 389(227) 389(227); UV 306, 284, 306, 284 nm.	82

(*Continued*)

Table 3. Continued

Compound determinated	Column	Mobile phase flow rate (mL/min)	Detection	Ref.
trans- and *cis* resveratrol-3-*O*-glucoside *trans*- and *cis*-resveratrol	Nova-Pak C_{18} (300 × 3.9 mm, 4 μm)	A. water/acetic acid (98:2, v/v), B. water/acetonitrile/ acetic acid (78:20:2, v/v), 0–80% B 0–55 min; 80–90% B 55–87 min, 90–90% B 57–70 min, 90–95% B 70–80 min, 95–100% B 80–90 min; washing with methanol and reequilibration of the column from 90–120 min 0.7 mL/min	t_R (min), λ_{max} $[M\text{-}H]^-$ [M-H-162], (*m*/*z*) 53.2,min 306 (s), 319 nm 227, 389; 68.9, min 285 nm, 227; 389 71.9, min 306,319(s), nm 227; 95.7, min 284 nm 227.	80
trans-resveratrol, standard	HP Innowas fused-silica capillary column (30 m × 0.25 mm df = 0.25 μm)	Direct-exposure-probe conditions	Ions produced by MS/CID experiments on resveratrol: $[M+H]^+$ m/z: 229, $[M+H-H_2O]^+$ m/z 211 and 135, 107	68

GE-gradient elution; ECD-electrochemical detector; APCI-atmosferic presure chemical ionization; ESI-electrospray ionization; ESI-electrospray ionization; SSI-sonic-spray-ionization; CID-collision-induced dissociation HBA-hydroxybenzoic acid; HCA-hydroxycinnamic acid; s-shoulder; t_R-retention time.

in (Table 3). Figure 1, shows two chromatograms with the direct injection sample of red wine, and after preparation of the red wine by L-L extraction procedure (Table 2). In the first case, the chromatogram is more complex and sensitivity for the determination of phenolic compounds is lower, (Figure 1a). However, in the second case, the complexity of the chromatogram was reduced, allowing separation with good resolution, detection, and identification of seventeen polyphenolic compounds (Figure 1b).

As it can observed from Figure 1, the chromatogram (Figure 1b) shows a bigger resolution, selectivity, and sensitivity compared with the chromatogram from (Figure 1a), because the sample matrix after L-L extraction becomes more simple. In conclusion, we can affirm that sample preparation of the wine allowed better identification and quantification of the compounds.

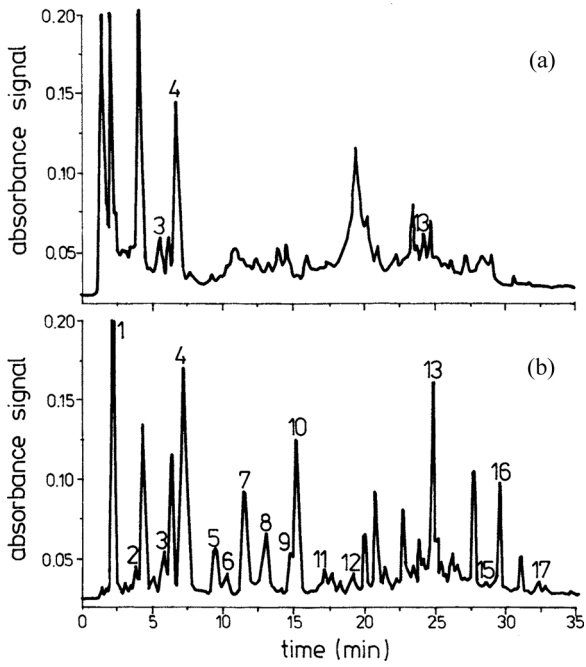


Figure 1. Chromatograms were performed by absorbance detection at $\lambda = 280$ nm. Directly injected sample red wine (a), and injected after L-L extraction procedure of the red wine (b). Sample peaks: 1. galic acid, 2. procatechuic acid, 3. protocatechuicaldehyde, 4. (+)-catechine, 5. 2,5-dihydroxybenzaldehyde 6. vanilic acid, 7. caffeic acid, 8. syringic acid, 9. (−)-epicatechin, 10. syringaldehyde, 11. *p*- cumaric acid, 12. feluric acid, 13. *trans*-resveratrol, 14. myricetin, 15. quercitrin, 16. quercetin, 17. kaempferol. (Adapted from [81])

Identification of Resveratrol and their Related Compounds

The identification of the compounds after separation is one of the most difficult steps of chromatographic analysis. For this purpose, a lot of methods that rely on comparison can be used:

- retention parameter (t_R),
- UV spectrum, and
- MS spectrum,

of the compound from the sample with a standard compound or spectral library.

Column strength in RP-HPLC can be defined in terms of the bonded phase, i.e., CN column being more weak than the C_{18} column, or polar and no polar column, respectively.

The elution in RP-HPLC with the C_{18} column is achieved in order of decreasing of the polarity of compounds: *trans*-, *cis*-piceid, *trans*- and *cis*-resveratrol.[82] Other examples show the same rule in order of elution of compounds: *trans*-astringin, *trans*-piceid, *trans*-, *cis*-resveratrol, ε-viniferin, and δ-viniferin,[58] and *trans*-, *cis*-resveratrol-3-*O*-glucoside, and *trans*-, *cis*-resveratrol.[80]

In the case of the CN column, order of elution will be reversed *vs.* the C_{18} column: *cis*-resveratrol glucoside, *trans*-resveratrol glucoside, *cis*-resveratrol and *trans*-resveratrol,[65] and *trans*-polydatin, *cis* polydatin.[66]

The UV spectra show a maximum of absorption for resveratrol and their derivatives: *trans*-resveratrol 305.6 nm,[55] 305.7 nm,[77] 306 nm, 319(s)[80] and *trans*-piceid, 317.5 nm,[55] and 306(s) nm 319 nm,[80] respectively, and for *cis*-resveratrol at 284.3 nm,[55] 287.8 nm,[77] 284 nm,[80] and *cis*-piceid at 285.5 nm,[55] 285 nm,[80] respectively. The use of a fluorescence detector in series with a UV absorbance detector allows increasing selectivity and sensitivity for the determination of the phenolic compound in wine samples.[81]

Published data on the molar absorptivity and chemical stability of *trans*- and *cis*-resveratrol have varied greatly. The *trans*-resveratrol is stable for months, except in high pH buffers, when it was protected from light, however, *cis*-resveratrol was stable only near pH neutrality when it was completely protected from light. Accurate values for UV absorbance for *trans*-resveratrol in ethanol solutions at $\lambda_{max} = 308$ nm was $\varepsilon = 30000$, and for *cis*-resveratrol at $\lambda_{max} = 288$ nm was $\varepsilon = 12600$.[64]

The mass spectrum is the same for the two isomers of resveratrol; however, the UV absorption spectra are clearly different, as can see above. Consequently, there would be a considerable error in quantifying *cis*-resveratrol by means of a calibration curve of the *trans*-resveratrol.[77]

Vian et al.[55] reported the separation and identification of four compounds: *trans*-piceid, *trans*-resveratrol, from the chromatogram of a Grenache wine at 306 nm, and *cis*-piceid, *cis*-resveratrol from same wine, but at 285 nm. The analyses were carried out using a LC-Waters (Miliford, MA, USA) equipped with a Model 600 pump and a Model 600 gradient controller connected to a Model 717 auto sampler and a Model 996 DAD. The chromatographic condition can be seen in Table 3.

The identification was performing by using the following criteria:

- retention time (*trans*-resveratrol 10–10.2 min, *trans*-piceid 5.5–5.7 min, *cis*-resveratrol 14.4–14.6 min, *cis*-piceid 11.7–12 min) and
- UV spectrum (Figure 2).

The florescence detection has good sensitivity and specificity and is being used in the most cases of wines analysis. Vitrac et al.[54] described

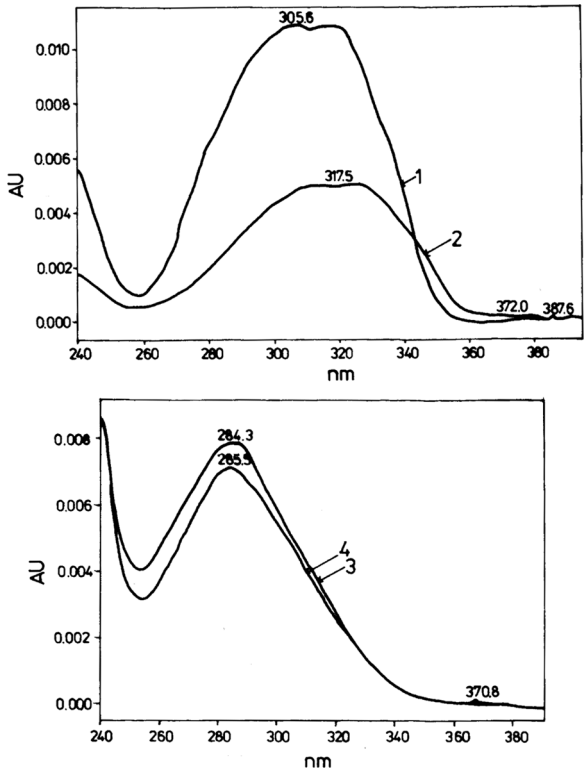


Figure 2. UV spectra: 1. *trans*-resveratrol, 2. *trans*-piceid, 3. *cis*-resveratrol and 4. *cis*-piceid. (Adapted from [55])

the determination of six stilbenes in wines by HPLC with direct injection and fluorescence detection. The analysis was performed in a Bischoff LC equipped with two pumps (Model 2250), an automated gradient controller (Normasoft software), and an automated injector (Alcott, Model 708). Detection was performed either with a programmable FLD (Groton, Model FD-500) or with a UV-Vis detector (Kontron, Model 430). Experimental conditions are presented in Table 3. Quantifications were performed at optimal wavelengths for each compound during separation, as can be seen in Figure 3.

Today, the HPLC-MS system is frequently used for separation, detection, and concomitant identification of compounds from wines. A fast method has been developed for the determination of total free resveratrol in wine extracts.[68] The experimental chromatographic conditions

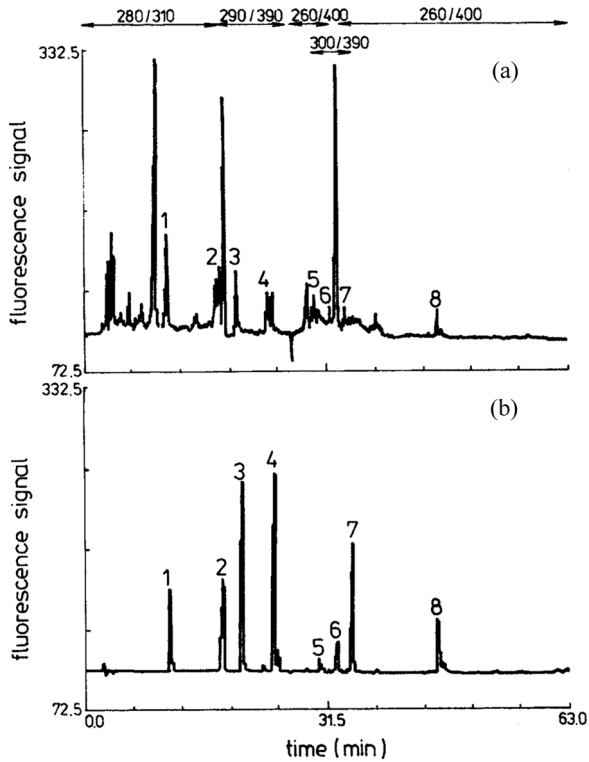


Figure 3. Chromatograms were obtained by fluorescence detection. The wavelengths changes are shown at the top of the figure. Direct injection sample of red wine (a) and standard mixture of polyfenols (b). Standard peak: 1. (+)-chatechin, 2. (−)-epichatechin, 3. *trans*-astringin, 4. *trans*-piceid, 5. *cis*-piceid, 6. *trans*-resveratrol, 7. pallidol, 8. *cis*-resveratrol. (Adapted from [54])

are shown in Table 3. The mass spectrometry experiments were performed with a ThermoFinningan PolarisQ ion trap mass spectrometer (Austin, TX, USA), operating in positive-ion chemical ionization (PICI) mode with methane as reagent gas. Another mode of operation was direct exposure probe (DEP) using a ThermoQuest (Austin, TX, USA), instrument equipped with a rhenium filament for analysis of thermally labile compounds. The sample was lodged on the filament and the solvent was evaporated gently by a warm air flow. For analysis of a standard solution, a heating program for the filament was used. The DEP/MS-SCAN mass spectrum of a resveratrol standard solution at a concentration of 100 mg/L, showed the $[M+H]^+$ ion that is clearly visible at m/z 229. The ion trap was operating simultaneously in normal scan, and MS/MS mode, respectively. MS/MS collision induced dissociation (CID) experiments on the selection of the $[M+H]^+$ ion of resveratrol at m/z 229 gave fragments $([M+H]\text{-}H_2O)^+$ at m/z 211, and other ions, as can be observed from Figure 4.

The nonanthocyanin phenolic compounds in red wines, were extracted and analyzed by HPLC-DAD/ESI-MS.[80] A quadruple mass spectrometer Hewlett-Packard series 1100, (Palo Alto, CA) with a electrospray ionization (ESI) interface was used for simultaneous determination of nonanthocyanin phenolic compounds in red wines. The ESI was operated in negative mode scaning from m/z 100 to 3000, using the following fragmentation program: m/z 0 to 200 (100 V) and from 200 to 3000 (200 V), respectively. In the case of the glucosides form of *trans*-, and *cis*-resveratrol, a fragment ion, $[M\text{-}H\text{-}162]^-$ corresponding to resveratrol after the loss of the glucose moiety was observed in addition to the molecular ion, $[M\text{-}H]^-$ m/z 227.

Mark et al.[51] have elaborated and validated a new, simple procedure for the quantitative analysis of *trans*-resveratrol and *trans*-piceid in wine. The analysis was performed using a Finigan AQA (ThermoQuest, San Jose, CA, USA) equipped with both APCI and ESI interface. Spectra were recorded in the negative ion mode between m/z 10 to 700. APCI negative ion MS spectra of *trans*-resveratrol shows a principal ion $[M\text{-}H]^+$ at m/z 228.9 and *trans*-piceid $[M\text{-}H]^+ m/z$ at 388.8, and a principal ion $([M\text{-glucose}]\text{-}H)^+$ m/z at 226.8, respectively. When operating in ESI, the negative ion *trans*-resveratrol gives a principal ion $[M\text{-}H]^+$ m/z at 227, and *trans*-piceid $[M\text{-}H]^+$ m/z at 338.7, $[M+Ac]^-$ m/z at 478.8, and a principal ion $([M\text{-glucose}]\text{-}H)^+$ m/z at 276.7, respectively.

Recently Buiarelli et al.[56] have developed a method for the direct determination of some stilbenes (*trans*- and *cis*-resveratrol, *trans*-and *cis*-resveratrol glucoside, *trans*-and *cis*-piceatannol, and *trans*-and *cis*-piceatannol glucoside) in Italian wines. Analysis was performed by HPLC/MS using a triple quadrupole (QqQ) PE-SCIEX API 356 (Perkin Elmer Sciex Instruments, Foster City, CA, USA), in multiple reaction

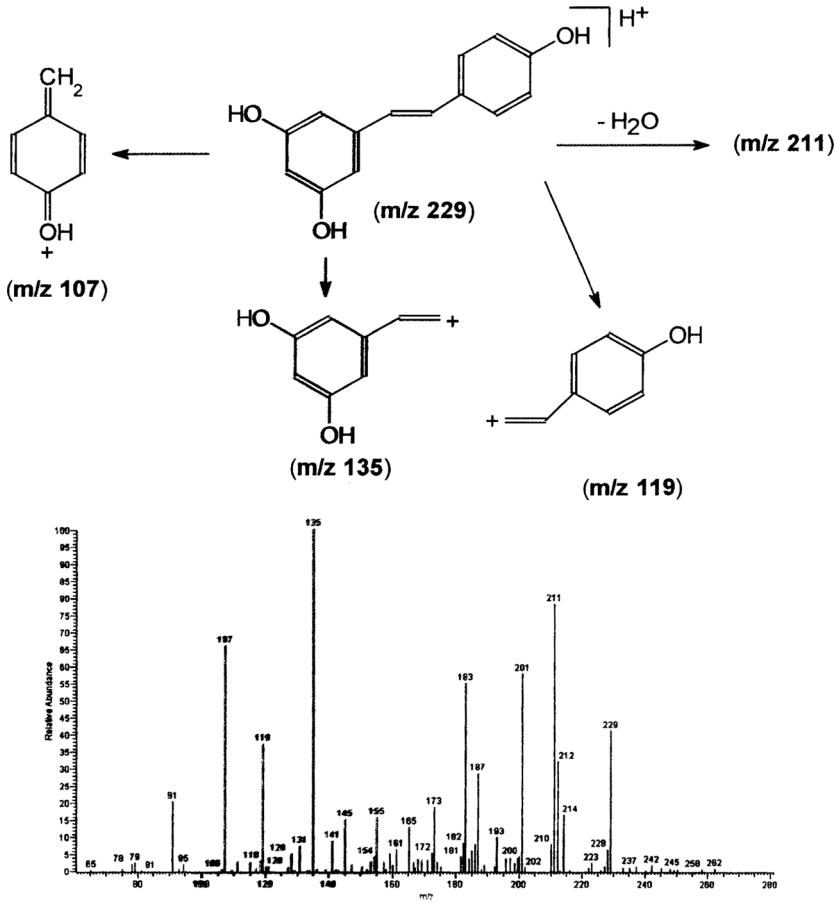


Figure 4. Principal ions produced by CID experiment on *trans*-resveratrol $[M+H]^+$. (Adapted from [68])

monitoring (MRM) mode, acquiring two diagnostic product ions from the chosen precursor. Operating by ESI in negative ion mode it gives higher sensitivity for all the target compounds than APCI. Negative ion ESI gave the best results, because of their acidity stilbenes are more efficiently ionized in negative ion mode and are easily deprotonated in the liquid phase. The chromatographic conditions are presented in Table 3.

The standard solution was prepared either in an aqueous solution of $HCOONH_4$ or HCOOH. The mass spectrum of *trans*-resveratrol is dominated by the $[M\text{-}H]^-$ ion m/z at 227.0, which confirms the molecular weight. However, the MS/MS product ion spectrum of m/z at 227.0

shows the two product ions at m/z 185.0 $[M\text{-}H\text{-}CH_2CO]^-$ and the more abundant ion at m/z 143.0 $[M\text{-}H\text{-}2CH_2CO]^-$, which involved the sequential loss of two ketone molecules, $H_2C=C=O$, being monitored by MRM analyses. The retention times, t_R, for *trans*- and *cis*-resveratrol are 25.4 min and 31.1 min, respectively.

The *trans*-resveratrol glucoside (*trans*-piceid) mass spectrum shows $[M\text{-}H]^-$ ion at m/z 389.2 and the format adduct $[M+HCO_2]^-$ m/z at 435.2. Furthermore, in MS/MS was observed an ion at m/z 227.2 (represent deprotonated molecule of *trans*-resveratrol), which derives from the loss of the glucoside moiety $[M\text{-}C_6H_{11}O_5]^-$; and t_R for *trans- and cis*-piceid are 17.1 min and 26.2 min, respectively.

The *trans*-piceatannol in the negative ion ESI-MS spectrum presented the molecular ion m/z at 243.0 $[M\text{-}H]^-$, and t_R 19,7 min. However, in ESI-MS/MS spectrum was observed the n$[M\text{-}H]^-$ ion m/z at 243.0, which easily confirms the molecular weight, and the fragment ions m/z at: 158.9, and 201.0 derived from successive losses of C_2H_2O.

For the identification of *trans*- astringin, MS (Q/TOF) was carried out. The negative ion TOF full scan ion spray mass spectrum of astringin in red wine samples are the following: $[M\text{-}H]^-$ m/z at 405.30 and a main fragment ion $[M\text{-}C_6H_{11}O_5]^-$ m/z at 243.18 and m/z at 359.20. The spectrum was obtained by combining the spectra acquired during the elution of the peak at t_R 12.10 min.

The resveratrol dehydrodimer (δ-viniferin) is one of the major stilbenes, which were produced in vitro by the oxidative dimerization of resveratrol by plant peroxidases or fungal laccases.[85] It was recently identified in wines and grape cell cultures. This dimmer was identified by HPLC-DAD-MS and structural by NMR. The mass spectra were performed by HPLC/MSD trap in ESI mode (Agilent 1100 series, Santa Clara CA USA). By this experiment, it was confirmed that ε-viniferin, $[M\text{-}H]^-$, m/z at 453.1 and δ-viniferin and $[M\text{-}H]^-$, m/z at 453.0 were *trans*-resveratrol dimmer, and the same results gave MS/MS spectra: $[M\text{-}H]^-$, m/z at 453.1 and 453.0, $([M\text{-}H]\text{-}H_2O)^-$ m/z at 435.1, 435.1, respectively.

The specificity of the detectors is: moderate for UV, better with DAD; good with fluorescence, and high with MS.

Limit of Detection and Quantification

Two important characteristics for the liquid chromatography method are the limit of detection (LOD) and limit of quantification (LOQ). The LOD is definite as the minimum detectable amount of analyte or, in other words, is the smallest concentration that can be detected reliably. The LOD is related to both the signal (S) and the noise (N) of the liquid

chromatographic system. Usually, LOD is defined as a peak whose signal to noise S/N ratio typically is 2 or 3. It is recommended that S/N ratio of 3 (signal 3, and noise 1) be used as the limit of detection for HPLC methods.

The LOQ can be defined as the smallest concentration of analyte, which gives a response with a specified level of accuracy and precision. The LOD can be defined in many ways. One method uses a similar technique to that for LOD but requires S/N ratio of at least 10 (signal 100, and noise 10). The range of a method can be defined as the lower and upper concentrations for which the analytical method has adequate accuracy, precision, and linearity.

The LOD and LOQ values that were determined are affected by the experimental conditions of the separation: column, elution conditions, detector, and especially chemical structure of the compound. Problems caused by differences in the wavelengths for maximum UV absorption of the individual compound can be resolved by using DAD.

Detection based on fluorescence is generally more sensitive with one order of magnitude than UV absorption, as can be observed from Table 4.

To demonstrate the difference between performance DAD *vs.* MSD, two papers were selected.[51,77] In these papers, the chromatographic conditions were absolutely identical, as can be discussed below.

For quantitative analysis of *trans*-resveratrol and *trans*-piceid in wine, a HPLC system with UV-vis and MS detection was used.[51] The HPLC system consisted of a Gynkotek M 580 GT pump, Rheodyne 8125 injector (20 μL loop) (Cotati, CA, USA), and a Gynkotek M 340S UV DAD (Gynkotek GmbH, Germering, Germany). HPLC-MS analysis was carried out using a Finnigan AQA (Thermo-Quest, San Jose, CA, USA) equipped with both APCI and ESI in negative ion. This system showed greater ionization stability and has been the method of choice for analysis. The chromatographic conditions were the same for both systems of detection. The LOD ($S/N = 3$) was 0.9 pmol for *trans*-resveratrol and 0.6 pmol for *trans*-piceid when was using UV-vis at 306 nm detection. However, when using MS detection, the LOD was 0.3 pmol and 0.2 pmol, respectively for ($n = 5$). In this case, the LOD obtained by MS is three times lower, *vs.* DAD.

A method has been developed for LOD determination of *trans*-, and *cis*-resveratrol by using a Waters Integrity HPLC-DAD-MS system (Waters, Millipore, Milford, MA, USA). The same separation conditions were used for both types of detectors. The detection by MS was performed in the selected ion monitoring (SIM) mode at m/z 228. This comparative study show the following values for *trans*-, and *cis*-resveratrol in MS-SIM mode 1.202 μg/mL and 2.558 μg/mL, respectively, and for DAD (305 nm) 0.948 μg/mL and (280 nm) 0.834 μg/mL, respectively.[77]

Table 4. Performance comparison of several detector types

Type of detector	Compound	LOD (μg/mL)	LOQ (μg/mL)	Range of linearity (μg/mL)	Ref.
Absorbance					
UV, 306 nm	*trans*-resveratrol	<0.03			43
	cis-resveratrol	0.100			
UV, 306 nm	*trans*-resveratrol	0.9 pmol (205 pg)			51
	trans-piceid	0.6 pmol (234 pg)			
UV, 306 nm	*trans*-resveratrol	0.032	0.10		55
285 nm	*cis*-resveratrol	0.034	0.11		
UV, 306 nm	*trans*-resveratrol	1.548			57
UV, 280 nm	*trans*-resveratrol	0.020			73
DAD 306 nm	*trans*-resveratrol	0.025			59
UV, 306 nm	*trans*-resveratrol	0.002	0.007		74
DAD	*trans*-resveratrol	0.030		0.4–8.5	92
DAD 280 nm	Catechin	0.20	0,67	1–25	63
280	epicatechin	0.16	0,53	1–25	
360	rutin	0.11	0,35	0.2–20	
300	*trans*-resveratrol	0.06	0,22	0.1–20	
300	*cis*-resveratrol	0.21	0,72	0.5–20	
360	quercetin	0.16	0,55	0.2–20	
UV 280 nm	*trans-astringin*	0.32[a] 8 ng			58
286 nm	*trans*-piceid	0.20; 5 ng			
306 nm	*trans*-resveratrol	0.20; 5 ng			
306 nm	*cis*-resveratrol	0.32; 8 ng			
321 nm	ε-viniferin	0.32; 8 ng			
321 nm	δ-viniferin	0.32; 8 ng			
UV 306 nm	*cis*-polydatin	0.20[b]; 0.075[c]			66
	trans-polydatin	0.11[b];0.048[c]			
DAD 305 nm	*trans*-resveratrol	0.948	3.16	2.50–50.	77
	cis-resveratrol	0.834	2.781	1.23–40.	
UV 280 nm	galic acid	0.06	0.19	0.7–7.2	81
	protocatechuic acid	0.12	0.39	1.0–10.4	
	protocatechuicaldehyde	0.03	0.10	0.6–6.4	
	(+)-catechin	0.11	0.36	3.4–33.6	
	vanilic acid	0.07	0.23	0.5–4.8	
	caffeic acid	0.06	0.20	0.4–4.4	
	syringic acid	0.05	0.16	0.4–4.4	
	(−)-epicatechin	0.34	1.13	3.6–36.4	
	Syringaldehide	0.04	0.13	0.7–6.8	

(*Continued*)

Table 4. Continued

Type of detector	Compound	LOD (μg/mL)	LOQ (μg/mL)	Range of linearity (μg/mL)	Ref.
	p-cumaric acid	0.08	0.26	0.4–4.0	
	feluric acid	0.08	0.27	0.5–5.6	
	trans-resveratrol	0.02	0.07	0.4–4	
	miricetin	0.06	0.20	0.6–6.0	
	quercitrin	0.51	1.60	2.5–23.6	
	quercetin	0.54	1.50	1.6–16	
	Kaempferol	0.21	0.70	1.5–15.2	
Florescence					
FL $\lambda_{ex}/\lambda_{em}$, nm					81
278/360	(+)-catechin	0.093	0.31		
278/360	vanilic acid	0.068	0.22		
278/360	(−)-epicatechin	0.003	0.01		
278/360	syringaldehide	0.031	0.10		
330/360	*trans*-resveratrol	0.003	0.01		
FL $\lambda_{ex}/\lambda_{em}$, nm					54
280/310	(+)-catechin	0.02	0.07	0.5–10	
280/310	(−)-epicatechin	0.01	0.05	0.5–10	
290/390	*trans*-astringen	0.01	0.06	0.5–10	
290/390	*trans*-piceid	0.01	0.05	0.1–10	
260/400	*cis*-piceid	0.03	0.1	1.0–10	
300/390	*trans*-resveratrol	0.01	0.03	0.5–10	
260/400	pallidol	0.02	0.03	0.5–10	
260/400	*cis*-resveratrol	0.02	0.07	1.0–10	
FL $\lambda_{ex}/\lambda_{em}$, nm, 360/374	*trans*-resveratrol	0.118			57
L $\lambda_{ex}/\lambda_{em}$, nm, 360/374	*trans*-resveratrol	0.003			73
FL $\lambda_{ex}/\lambda_{em}$, nm					63
280/315	(+)-catechin	0.003	0.01	0.01–1	
280/315	(−)-epicatechin	0.005	0.02	0.03–1	
324/370	*trans*-resveratrol	0.003	0.01	0.01–1	
260/370	*cis*-resveratrol	0.001	0.004	0.01–0.1	
Chemoluminescence					
CL	luminal/potassium ferricyanide/*trans*-resveratrol	0.000166 (70 pmol)		0.0005–0.750	59
Mass spectrometry					
MS with APCI, and ESI negative ion	*trans*-resveratrol	<0.3 pmol (68 pg)			51

(*Continued*)

Table 4. Continued

Type of detector	Compound	LOD (μg/mL)	LOQ (μg/mL)	Range of linearity (μg/mL)	Ref.
	trans-piceid	<0.2 pmol (78 pg)			
MS	*trans*-resveratrol	1.202	4.005	2.5–50	77
	cis-resveratrol	2.558	8.525	1.23–40.06	
MS	*trans*-resveratrol	0.048			57
MS	*trans*-resveratrol	200 pg			76
TOFMS, ESI	*trans*-resveratrol	0.048	0.160	0.2–6.0	56
	trans-piceid	0.048	0.160	0.2–40.0	
	trans-piceatanol	0.050	0.167	0.2–6.0	
MS/MS with APCI negative ion	*trans*-resveratrol	0.005	0.026	0.026–1.675;	60
	cis-resveratrol	0.005	0.022	0.012–1.460	
Electrochemical					
EC; + 0.75 V	*trans*-resveratrol	0.003			43
	cis-resveratrol	0.015			

[a]Detection limit calculated according to IUPAC rules (25 μL).
[b]isocratic.
[c]GE gradient elution.

The application of LC-ESI-MS/MS was investigated for the analysis of *trans*-resveratrol in red wine, grape skin, grape pomace, and wine-making by products. The LOD was calculated in the low part per billion range (10 μg/L). These results were compared with those obtained using an LC-UV/DAD method.[86]

Electrochemical detection is a particularly useful method for determination of electroactive compounds, such as phenols, with better sensitivity than UV detection. The value of + 0.75 V was considered the best potential with very good selectivity. The detection limit ($S/N = 3$) was 0.003 μg/mL for *trans*-resveratrol and 0.015 μg/mL for *cis*-resveratrol.[43]

CONTENT OF STILBENES IN WINE

Grapes are probably the most important source of resveratrol for humans, since the compound is also found in one of the end products of grape wine. Resveratrol is found in all variety of wines, but the highest amount is found in red wines.

The interest of the scientific community in the resveratrol and related compounds in the wine has increased over the last years. Several reviews

on resveratrol content in different varieties of wines from different regions of world was published.[87–91]

Stervbo et al.[87] published a review in connection with resveratrol content in red wines based on relevant published data. Red wine contains an average of 1.9 ± 1.7 mg/L *trans*-resveratrol, ranging from non-detectable levels to 14.3 mg/L *trans*-resveratrol. The average level of *trans*-resveratrol-glucoside (*trans*-piceid) in red wine may be as much as 29.2 mg/L. The resveratrol monomers (*trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, and *cis*-piceid) were identified and quantified in Spanish white and rose wines. The white wines had levels between 0.051 and 1.801 mg/L. Rose wines had a total average level of 2.15 mg/L for resveratrol isomers. This value is situated between those of red and white wines.[88] Furthermore, a short review is focused on direct analysis of food samples and wines by HPLC.[89] The importance of sample preparation in determination of phenolic compounds in plant materials, particularly fruit and wine was resumed in a large review.[90] A comparison of published methods of HPLC analysis for resveratrol and other related phytoalexins in peanut extract and wines were summarized, and a RP-HPLC method for analyzing was developed.[91]

A group of fifty two commercial wines from different Italian regions, cultivars, and winemaking technologies, was analyzed by HPLC-UV-DAD, in order to assess the influence of several parameters on the *trans*-resveratrol concentration.[57] The information obtained by this study was that amounts of *trans*-resveratrol in red wines range from 0.32 to 8.29 mg/L, however, white wines have a lower amount of *trans*-resveratrol, not always quantifiable. The conclusion was that this difference in *trans*-resveratrol concentration is certainly due to the different winemaking procedure. Also, the quantitative data for twenty two Italian types of grape variety wines obtained by HPLC-MS/MS analysis was reported.[56] In particular, piceid has been shown to be the stilbene present at the highest concentration, more in *cis*-form ranging from a minimum of 0.82 mg/L for a white wine, Pinot Grigio, to a maximum of 38.87 mg/L for Montepulciano 3. Appreciable amounts of *trans*-, and *cis*-resveratrol, have been determined in red wine samples, ranging from 0.63 to 3.39 mg/L, and from non-quantifiable to 4.93 mg/L, respectively.

Eighteen monovarietal red wines[37] and twenty-six varietal white wines[38] were analyzed from different Spanish appellations and vintages. Total resveratrol content has been quantified in a survey of forty five Monastrel monovarietal Spanish red wine types as glucoside and aglycone forms of resveratrol.[39] Furthermore, when wines were made using macerative fermentation with dabble amounts of solid parts, "double pasta" reached the highest levels of total stilbene content expressed as resveratrol equivalent, i.e., 30 mg/L with an average of 18.8 mg/L. The

Manastrell variety can be characterized by high resveratrol glucoside concentration and low free isomer content. In fifty-eight bottle red wines from the 1999 harvest, belonging to seven denominations of Origin of the Canary Island (Spain) wine the content of *trans*-resveratrol was determined.[73] The average level found in bottled red wines was 2.89 mg/L. Also, *trans*-resveratrol content was similar in all red wines commercially available from the Canary Islands with an average value of 3.00 ppm.[81]

In a survey of 120 commercial wines from Portugal and France, the highest concentrations of stilbenes were found in red wines.[42] The resveratrol contents by HPLC in a number of 98 commercial wines from Aragon, from several vintages, were analyzed.[44] Concentration of *cis*-resveratrol ranged between 0.20–5.84 mg/L in red wines and between 0.02–3.17 mg/L in rose wines, and for *trans*-resveratrol levels ranged between 0.32–4.44 mg/L in red wines and between 0.12–2.80 mg/L in rose wines. French red wines are an abundant source of phenolic compounds, especially catechins.[45]

A lot of the Greek wines (eighteen white and eighteen red varietal wines) were analyzed for their content of *trans*-resveratrol. As it is known, red wines were found to have higher concentrations of *trans*-resveratrol between 0.352–1.99 mg/L compared to with those obtained from white varieties 0.005–0.57 mg/L.[47] The concentration of the *trans*-resveratrol in wines from Greek markets was determined. Generally, the wines were not from a single vintage; no conclusion can be made for the role of age regarding the content of the *trans*-resveratrol in Greek wines.[46] A rapid HPLC method has been developed for the determination of *trans*-resveratrol in twenty nine red Greek wines of appellation of origin. The concentrations found varied between 0.550–2.534 mg/L.[74]

The levels of *trans*-resveratrol in thirty six commercial red wines produced in the southern region of Brazil have been determined by isocratic UV-HPLC methods. The concentrations of *trans*-resveratrol were found in the range from 0.82 to 5.75 mg/L with a mean value of 2.57 mg/L. The results indicate that these wines contain significant levels of *trans*-resveratrol.[48]

In a relative recent paper,[49] sixty-seven red wines from Hungarian Villany region, of vintages from 1996 to 2003, were analyzed for their polyphenolic composition by HPLC. The *trans*-resveratrol content levels were found between 1.1–3.1 mg/L with a mean of 2.3 mg/L. Another new, and simple method for the determination of phenolic compounds such as resveratrol and piceid isomers from seventy Hungarian red wines and three white wines from two wineries, has been elaborated and validated.[51] *Trans*-resveratrol and *trans*-piceid were found in red wines between 0.1–14.3 mg/L and 3.8–16.4 mg/L concentrations, respectively.

A group of fifteen commercially red and five white wines from different Romanian regions were studied. The concentrations of

trans-resveratrol found in red wines are in the range from 0.803–8.807 mg/L and for *cis*-resveratrol are in the range from 0.385 to 3.285 mg/L.[60]

The *trans*-resveratrol concentration in musts and wines produced from seven red and four white grape cultivars from wine growing regions of Turkey, were analyzed by HPLC methods.[53] The level of *trans*-resveratrol for white wines and must were found from 0.116–1.931 mg/L and from 0.0082–0.291 mg/L concentrations, respectively. In the case of red wine and must samples, the concentrations are in the range from 0.176–4.403 mg/L and from 0.0004–0.102 mg/L, respectively.

The mean of total phenolic acid concentrations in Niagara (Ontario, Canada) wines are situated in a narrow range from 0.200 mM/L (Gamay Noir) to 0.250 mM/L (Pinot Noir).[71] In this study, of the hydroxystilbene components, Pinot Noir wines were by far the highest polydatin concentrations, whereas their concentrations of free resveratrol isomers were among the lowest.

The forty-two vines produced from grapes grown in the Snake River Valley of Idaho USA, were examined.[93] The samples examined were from four monovarietal wines. *Trans*- and *cis-piceid* and resveratrol were found in the samples. Free stilbene levels ranging from 0.97 for Riesling wine to 12.88 for Cabernet Sauvigon wines were expressed as *trans-resveratrol* in mg/L. Phenolic content was determined in muscadine grapes by HPLC with fluorescence detection and allowed separation and detection of ellagic acid from resveratrol.[94] Contrary to previous results, ellagic acid in the skins (16.5 mg/100 g) and not resveratrol (0.1 mg/100 g) was the major phenolic in muscadine grapes.

CONCLUSIONS

The interest of the scientific community in the determination of stilbenes in wine by HPLC with modern detectors has increased over the last years. A large number of examples reported in this review shows that RP-HPLC with gradient elution is the preferred method. There are a lot of studies concerning the determination of LOD and LOQ for the separation of resveratrol and their related compounds by HPLC with several types of detectors. Several rapid methods by HPLC-MS for analysis of resveratrol have been published, also. The highest levels of *trans*-resveratro measured are: for red wines from 2.53 to 14.30 mg/L with an average of 5.72 mg/L; and for *cis*-resveratrol from 3.29 to 5.84 mg/L with an average of 4.69 mg/L. The average level of *trans* resveratrol for rose wines is 2.47 mg/L and for white wines 1.37 mg/L. Generally, levels of *cis*-resveratrol follow the same pattern as *trans*-resveratrol. The average level of *trans*-resveratrol-glucoside (*trans*piceid) in a red wine may be three

times of that of *trans*-resveratrol. The level of resveratrol varies from region to region and from one year to another.

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