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Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography

D.M. Goldberg[a,*], E. Ng[b], A. Karumanchiri[b], J. Yan[b], E.P. Diamandis[a], G.J. Soleas[c]

[a]*Department of Clinical Biochemistry, University of Toronto, Banting Institute, 100 College Street, Toronto, Ont. M5G 1L5, Canada*

[b]*Quality Assurance Department, Liquor Control Board of Ontario, Toronto, Ont., Canada*

[c]*Andres Wines Ltd., Grimsby, Ont., Canada*

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Abstract

We have developed a normal-phase high-performance liquid chromatographic method to measure the glucosides of *cis*- and *trans*-resveratrol as well as the free isomers in commercial wines. The procedure utilises a novel technique for calibration requiring only the *trans*-resveratrol in pure form and the conversion of the glucosides to the free isomers by hydrolysis with β-glucosidase. Only 20 μl of sample is needed for direct injection without prior preparation, and isocratic elution separates the 4 compounds of interest in approximately 50 min. Calibration curves are linear, and stable for several months. Recovery was around 100% for all compounds, and replicate analyses gave coefficients of variation of 0.4–6.7%. The limits of sensitivity were 0.2 and 0.1 μmoles l^{-1} for the glucosides and free isomers of *cis*- and *trans*-resveratrol, respectively. A preliminary survey of sixteen wines from eight countries or regions revealed the presence of all four compounds in fourteen; the glucosides of resveratrol were present in higher concentrations than the free isomers in five of the wines.

1. Introduction

Resveratrol (3,5,4'-trihydroxystilbene) exists in two isomeric forms. The *trans*-isomer occurs in the berry skins of most grape cultivars and its synthesis is stimulated by UV light, injury and fungal infection [1–5]. The *cis*-isomer has not been reported in any component of *Vitis vinifera*, but both isomers are present in variable amounts in commercial wines. Red wines contain much higher concentrations than white wines, presumably due to more prolonged skin contact during fermentation [6–8]. Certain cultivars (e.g., Pinot noir) have much higher concentrations than others (e.g., Zinfandel), and the region of growth may profoundly influence resveratrol concentrations of red wines, especially those from Cabernet Sauvignon [9].

Resveratrol is a precursor of the viniferins which are potent anti-fungal agents synthesized naturally by vines. In addition, it is a constituent of oriental folk medicines which have been employed in the treatment of heart and lipid disorders [10,11]. For this reason, it has evoked interest as a potential anti-atherosclerotic agent which may be responsible, at least in part, for

* Corresponding author.

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the decreased cardiac mortality rates observed in wine-consuming populations [12–14]. A number of investigations into the resveratrol concentrations of commercial wines have been conducted. These have mainly utilized methods for the measurement of the *trans*-isomer [6,7,9,15,16], but more recently methods to assay the *cis*-isomer have also become available [8,17]. The problem with the latter is the lack of a suitable standard. The only published procedures to synthesize resveratrol yield the stable *trans*-isomer as the main product [18,19], and only this isomer is commercially available at the present time. We have overcome this limitation by developing conditions permitting the predictable and quantifiable conversion of *trans*- to *cis*-resveratrol and have applied this principle to calibrate a HPLC method to assay both isomers without the need for any prior extraction steps. During this work, we recognized the presence in most commercial wines of two stilbene glucosides: polydatin (piceid), the 3-β-glucoside of *trans*-resveratrol, which shares some of its biological effects; and the corresponding glucoside of *cis*-resveratrol. We have adapted the method to measure these compounds as well.

2. Experimental

2.1. Liquid chromatography

A ternary HPLC (Spectra-Physics, San Jose, CA, USA) coupled to an SP 8000 pump, SP 8875 autosampler and Chrom Jet integrator was used with a Lichrosphere 100 CN (5 μm) column (250 × 4 mm I.D.) (Merck, Darmstadt, Germany) in the normal-phase mode. The mobile phase was water–acetonitrile–methanol (90:5:5, v/v) and the flow-rate (isocratic) was 1 ml/min. Untreated wine (20 μl) was directly injected and the absorbance was monitored at 306 nm using a 2550 variable-wavelength UV detector (Varian Instruments, Mississauga, Ont., Canada). In some experiments, complete spectra of the peaks of interest were obtained using a HP 1050 Series HPLC with diode-array detector and HPCL^{3d} Chem Station (Hewlett-Packard, Mississauga, Ont., Canada). Purity checks were performed by measuring the absorbance at 3 points on the spectrum (upswing, peak and downswing), and the ratios for unidentified peaks were compared with those of pure compounds using software provided by the manufacturer which calculated a quantitative estimate of spectral identity.

2.2. Calibration

Trans-resveratrol was purchased from Sigma (St. Louis, MO, USA) and dissolved in 96% ethanol (by volume) in a stock solution of 5 mmol l^{-1}. This was stable for at least 12 weeks at 4°C when protected from light. Working solutions were prepared in 0.2 *M* phosphoric acid–acetonitrile (4:1, v/v) to cover the range 2–100 μmol l^{-1}.

For *cis*-resveratrol calibration, aliquots of the *trans*-resveratrol stock were diluted into 0.2 *M* phosphoric acid–acetonitrile (4:1, v/v) to cover the range 4–300 μmol l^{-1}. A portion of each standard was placed in a UV box (Chromato-Vue, Ultraviolet Products, San Gabriel, CA, USA) and irradiated for 5–10 min at 254 nm and intensity of 990 $\mu W/cm^2$. Whereas the non-irradiated standard only contained the single peak of *trans*-resveratrol, this peak was diminished upon irradiation and was preceded by an earlier peak which was shown to be *cis*-resveratrol in an amount identical to the decrease in the *trans*-isomer. No other peaks were evident under these conditions. Values for the *cis*-resveratrol standards were therefore assigned on the basis of the decrease in *trans*-resveratrol following irradiation.

2.3. GC–MS

This was performed by direct injection of 1-μl samples of HPLC eluates or hydrolysed fractions using the following instrumentation: a Hewlett-Packard GC Model 5890 with quadrupole MS Detector (Model 5970) was coupled through a DB-17 ht column (J&W Scientific, Folson, CA, USA), 15 m × 0.25 mm I.D. and 0.15 μm film thickness. The complete analytical details and

mass spectral properties of underivatised *trans*- and *cis*-resveratrol have been published [17,19].

2.4. Enzymatic hydrolysis

β-glucosidase (Cat. No. G0395, 6.9 units/mg protein) and α-glucosidase (Cat. No. G7256, 87 units/mg protein) were purchased from Sigma. In accordance with a previous publication [20], these were added in a concentration of 2 mg per ml of untreated wine adjusted to pH 6.0 with a drop or two of 2 *M* NaOH, or to HPLC column effluents. Determinations of pH were made with the HI 9214 pH-Stick Meter (Hanna Institute, purchased from Fisher Scientific, Ottawa, Canada). The mixtures were then incubated at room temperature for 16–18 h (overnight).

3. Results

3.1. Development of the HPLC method

In varying the elution conditions to achieve optimal resolution of the *cis*- and *trans*-isomers of resveratrol, our attention was drawn to two peaks which eluted much earlier and which were variably present in many red wine samples. A typical chromatogram with the peaks of interest labelled A, B, C, D is illustrated in Fig. 1.

Peak D was identified as *trans*-resveratrol by the following criteria: (a) its retention time was identical to that of the pure *trans*-resveratrol standard; (b) its UV spectrum (Fig. 2) was identical to that of *trans*-resveratrol [6,21] and its purity was confirmed as >99% by diode-array analysis of the spectrum; (c) its mass ion spectrum determined by direct-injection GC–MS was identical to that of *trans*-resveratrol (Ref. [19] and Fig. 3) and it appeared to be 100% pure since no other ion fragments were detected.

Peak C was identified as *cis*-resveratrol by the following criteria: (a) it co-chromatographed with the new peak which appeared after brief UV irradiation of the pure *trans*-resveratrol standard and which we have previously characterized by GC–MS analysis of both derivatised and underivatised preparations as *cis*-resveratrol [17,22]; (b) its UV spectrum (Fig. 2) was consistent with that published by Siemann and

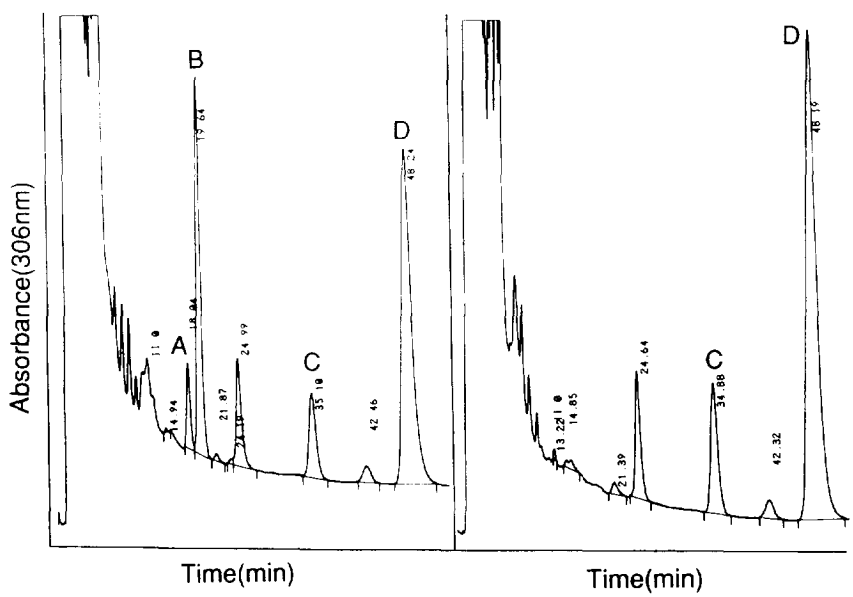

Fig. 1. Chromatograms of red wine directly injected into the HPLC. The left-hand trace is that of an untreated sample showing peaks A (*cis*-resveratrol glucoside), B (*trans*-resveratrol glucoside), C (*cis*-resveratrol), D (*trans*-resveratrol). The right-hand trace is that of the same wine treated with β-glucosidase (see Experimental) for 12 h, showing disappearance of peaks A and B, and increase in peaks C and D. Full-scale absorbance was 0.010 with attenuation of 4.

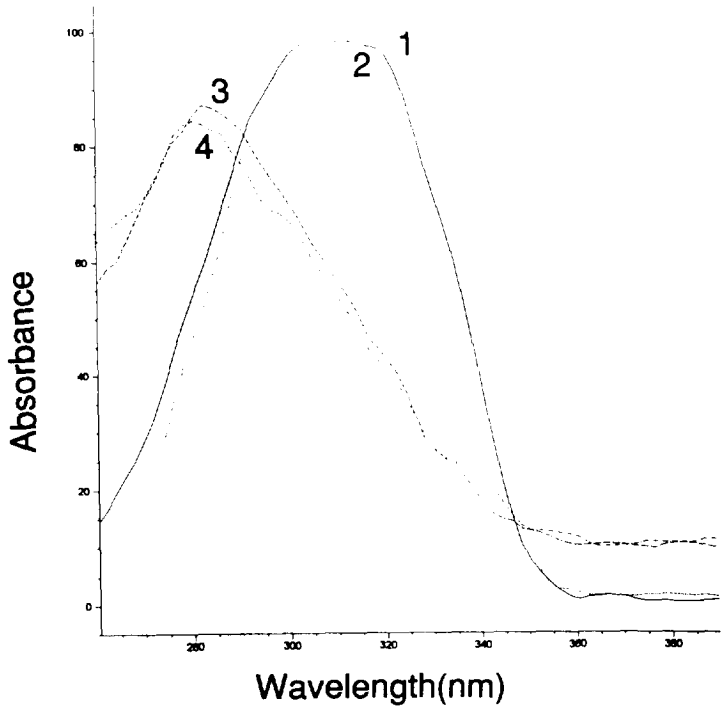

Fig. 2. Spectra of glucosides and free isomers by diode array detection: 1 = *trans*-resveratrol glucoside; 2 = *trans*-resveratrol; 3 = *cis*-resveratrol glucoside; 4 = *cis*-resveratrol.

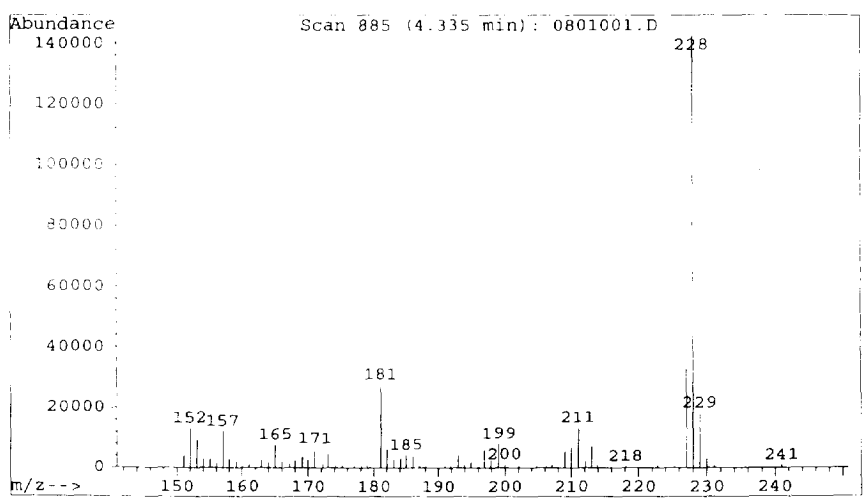

Fig. 3. Total-ion scan of peak A hydrolysed by β-glucosidase (see Experimental) for 12 h and subjected to direct-injection GC–MS (Ref. [19]). The molecular cluster at mass 229 [M^{+1}], 228 [M] and 227 [M^{-1}] typical of resveratrol and at the retention time characteristic of the *cis*-isomer (4.3 min) is clearly seen. An identical mass ion spectrum but at a retention time of 5.7 min (characteristic of *trans*-resveratrol) was obtained when the same procedure was carried out on peak B.

Creasy for *cis*-resveratrol [6] and its purity was confirmed as >99% by diode-array analysis of the spectrum; (c) its mass ion spectrum determined by underivatised direct-injection GC–MS [19] was identical to that of *trans*-resveratrol as presented in Fig. 3.

Peaks A and B were identified as the β-glucosides of *cis*- and *trans*-resveratrol, respectively, by the following criteria: (a) on treatment of the samples with β-glucosidase, there was a time-dependent decrease in these peaks accompanied by a proportional increase in peaks C and D until the former completely disappeared (Fig. 1); (b) α-glucosidase had no effect on these peaks; (c) the UV spectrum of peak A (Fig. 2) was nearly identical with that of peak C (*cis*-resveratrol), while that of peak B (Fig. 3) provided a close match to the UV spectrum of peak D (*trans*-resveratrol); (d) the peaks were collected independently, hydrolysed with β-glucosidase, extracted with ethyl acetate, and submitted without derivatisation to direct-injection GC–MS analysis [19] which for both peaks revealed the characteristic mass ion spectrum of resveratrol (Fig. 2) but the retention times of peaks A and B were identical to those of *cis*- and *trans*-resveratrol, respectively; (e) free glucose was identified in both peaks after hydrolysis by means of a specific enzymatic reaction [23]; (f) polydatin and *trans*-resveratrol were extracted from the dried roots of *Polygonum cuspidatum* by a published method [20], characterized by HPLC analysis, and on addition to an untreated wine sample co-chromatographed with peaks B and D, respectively, there being no *cis*-resveratrol or its derivatives in the *Polygonum* extract.

3.2. Interconversion of resveratrol isomers

On exposure of pure *trans*-resveratrol to UV irradiation for periods between 3 and 5 min, partial conversion to *cis*-resveratrol occurred without destruction of either isomer or the appearance of other products. When the mixture was heated in a water bath at 70°C for 30 min, substantial reconversion of *cis*- to *trans*-resveratrol took place without loss of either isomer. Table 1 illustrates these events over a series of irradiation–heating cycles. The amount of *cis*-resveratrol generated in step 1 was deduced from the reduction in the amount of *trans*-resveratrol. Proportionality between peak areas (counts at 306 nm) and resveratrol concentrations was assumed for both isomers in the subsequent steps.

Table 1
Evidence for quantitative interconversion of *trans*- and *cis*-isomers of resveratrol by UV irradiation and heat treatment

Treatment	Peak area at 306 nm (arbitrary units × 10^{-6}) peak C[a]	*cis*-Resveratrol concentration (μmol l^{-1})	Peak area at 306 nm (arbitrary units × 10^{-6}) peak D[a]	*trans*-Resveratrol concentration (μmol l^{-1})
None	0	0	9.52	302
UV, 10 min	1.90	191	3.46	111 (−91)
70°C, 20 min	1.31	133 (−60)	5.27	168 (+57)
UV, 10 min	2.49	253 (+120)	1.48	48 (−120)
70°C, 25 min	2.02	205 (−48)	3.08	99 (+51)
UV, 5 min	2.48	252 (+47)	1.49	49 (−50)
70°C, 35 min	1.50	152 (−100)	4.60	147 (+98)

Trans-resveratrol, 302 nmol l^{-1} in 0.2 *M* phosphoric acid–acetonitrile (4:1, v/v), was analysed by HPLC. The solution was then treated to successive episodes of UV irradiation and heating as described, and analysed by HPLC after each cycle. The relationship between peak area at 306 nm and *cis*-resveratrol concentration was based on the assumption that all of the *trans*-resveratrol lost during the first irradiation cycle was converted quantitatively to *cis*-resveratrol. The proportionality factors relating peak area to concentration for each isomer were used to calculate the actual concentrations after each treatment cycle (open data). The loss or gain of each isomer (in parentheses) demonstrated virtually 100% recovery throughout the 6 treatment cycles. This experiment was repeated several times with similar results.

[a] The peaks C and D correspond to *cis*- and *trans*-resveratrol, respectively (Fig. 1).

With this assumption, the changes in isomer concentrations balanced almost exactly such that the sum of the isomers equalled the initial concentration of *trans*-resveratrol. Proof of validity was also provided by direct-injection GC–MS analysis of the irradiated and heated preparations when results in good agreement with those of Table 1 were obtained showing that the sum of the two isomers at each step was equal to the initial concentration of *trans*-resveratrol. Known concentrations of *cis*-resveratrol for the purpose of calibration can thus be generated by irradiating pure standards of *trans*-resveratrol under the conditions defined in this paper and measuring its residual concentration; the difference represents the concentration of *cis*-resveratrol in the mixture. This procedure requires neither pure *cis*-resveratrol or GC–MS facilities to accurately quantitate the latter.

3.3. Calibration and recovery

Calibration curves for both *trans*- and *cis*-resveratrol were prepared as described in Section 2.2 and showed excellent linearity for both isomers (Table 2). Moreover, the curves remained constant over at least 4 months, or for the working life of the column (approximately 1000 injections), so that it was unnecessary to construct a complete calibration curve with each batch of samples. Routinely, the calibration is checked by running in duplicate a standard wine containing each isomer prior to a batch of unknown samples. Recalibration is considered only if the mean difference between the known and calculated values exceeds 5%.

Using this calibration procedure, recovery was determined by adding aliquots of irradiated *trans*-resveratrol standards of varying defined composition to 10 previously analyzed red wines and repeating the analyses. The data showed mean recoveries of 105% and 100% for *trans*- and *cis*-resveratrol, respectively, over the ranges 12.2–54.8 μmol l^{-1} and 14.4–67.6 μmol l^{-1}, respectively.

To calibrate the glucosides of resveratrol, eluates containing only peak A or peak B were pooled from a number of wines in which these were present in high amounts. The pooled eluates were concentrated and a series of dilutions were prepared. Each dilution was divided into two aliquots, one of which was run through the HPLC to record the absorbance at 306 nm and to confirm that only a single glucoside was present. The other aliquot was treated with β-glucosidase and then subjected to HPLC to quantitate the resveratrol isomers, of which only the appropriate form was present. Thus, there was no contamination of the pooled peaks and no isomerisation of the resveratrol released by enzymatic hydrolysis. Curves were constructed relating absorbance of the glucoside peaks to the molar concentration of the corresponding resveratrol isomer (Table 2). These showed excellent linearity with correlation coefficients (r) >0.980. As with the calibration curves for *trans*-

Table 2
Statistical evaluation of linearity of calibration data for the glucosides and isomers of resveratrol with the present HPLC method[a]

Compound	Concentration range (μmol l^{-1})	Slope (SE) arbitrary units[b] (μmol l^{-1})	Intercept (SE) arbitrary units[c]	P	r
cis-Glucoside	0.22–15.79	11863 (299)	−1967 (2697)	0.50	0.998
trans-Glucoside	0.13–8.20	38344 (1681)	4041 (7919)	0.62	0.983
cis-Resveratrol	2.98–62.90	11244 (132)	1322 (8213)	0.88	0.999
trans-Resveratrol	0.14–58.60	36256 (201)	−44116 (22855)	0.08	0.999

[a] Regression analysis was conducted according to the equation: y(arbitrary units) = m(slope, arbitrary units, μmol l^{-1})x (concentration, μmol l^{-1}) + C(intercept, arbitrary units).
[b] The slopes were in all instances significantly different from zero at a level of $p < 0.0001$.
[c] The intercepts were not significantly different from zero in any instance.

and *cis*-resveratrol, these were stable over several months so that the same strategy to check *trans*- and *cis*-calibration was employed. In practice, aliquots of a single wine containing all four peaks were stored at 4°C in the dark, tightly stoppered, and one aliquot was analysed in duplicate at the start of each routine batch. After 6 weeks, a new set of aliquots from a freshly opened bottle was prepared. The isomers and glucosides of resveratrol were perfectly stable under these conditions.

Recovery of the glucosides was tested by mixing three wines in which they were present in relatively high amounts with three wines in which they were low or absent and analysing their concentrations in the mixture (in quadruplicate). The results demonstrated average recoveries of 106% and 102% for the *cis*- and *trans*-glucosides, respectively.

3.4. Detection limit and precision

The detection limit was measured as the concentration corresponding to the lowest signal above baseline that reproducibly reached statistical significance at $p < 0.01$. By this criterion, the values for *cis*-resveratrol and its glucoside were 0.20 and 0.30 μmol l^{-1}, respectively, and for *trans*-resveratrol and its glucoside 0.11 and 0.16 μmol l^{-1}, respectively. Precision was evaluated by performing 10 replicate analyses within the same working day on two wines differing approximately two-fold in their concentrations of the four compounds of interest. The coefficients of variation ranged from 0.4 to 3.9% with the exception of *cis*-resveratrol glucoside at the lower concentration (6.7%).

3.5. Assays on wines

Two commercial wines from each of eight countries or regions were analysed (Table 3). All contained measurable and frequently high concentrations of both glucosides and the two free isomers of resveratrol, exceptions being two wines in which the *trans*-glucoside concentration was below the limit of detection. In five wines, the sum of the glucosides exceeded the sum of the free isomers, and in only five was the former sum less than half that of the latter. In nine of

Table 3
Concentrations of resveratrol isomers and glucosides in some commercial red wines (μmol l^{-1})

Vintage	Country/ region	Glucoside			Resveratrol		
		cis	*trans*	Total	*cis*	*trans*	Total
1993	Côtes du Rhône (France)	4.9	7.2	12.1	5.8	9.8	15.6
1993	Côtes du Rhône (France)	8.6	7.7	16.3	5.7	10.3	16.0
1993	Burgundy (France)	13.8	7.7	21.5	12.4	20.2	32.6
1992	Burgundy (France)	4.5	0.7	5.2	10.7	6.2	16.9
1991	Bordeaux (France)	1.7	3.2	4.9	17.5	33.4	50.9
1990	Bordeaux (France)	1.7	4.7	6.4	3.4	15.4	18.8
1991	Spain	3.6	14.6	18.2	0.1	9.2	9.3
1991	Spain	8.8	5.6	14.4	1.6	5.0	6.6
1990	Chile	0.6	2.2	2.8	0.2	3.6	3.8
1992	Chile	5.4	5.2	10.6	3.8	6.9	10.7
1992	Oregon (USA)	12.3	0.8	13.1	13.0	1.4	14.4
1992	Oregon (USA)	8.0	ND	8.0	10.9	7.3	18.2
1992	Niagara (Canada)	6.5	ND	6.5	14.9	25.4	40.3
1991	Niagara (Canada)	28.6	9.3	37.9	3.1	5.3	8.4
1992	California (USA)	0.9	2.0	2.9	0.3	2.0	2.3
1991	California (USA)	2.6	2.6	5.2	2.1	4.5	6.6

ND = not detected.

the sixteen wines the concentration of the *cis*-glucoside exceeded that of the *trans*-glucoside, whereas the concentration of the *trans*-resveratrol was greater than that of *cis*-resveratrol in fourteen.

4. Discussion

In the last few years, several methods to measure *trans*-resveratrol have been presented. These include a sensitive radioimmunoassay using "in house" reagents [24], GC [8], and GC–MS methods [19,21]. However, HPLC assays have been the most frequently employed due to the widespread availability of the required instrumentation [6,7,15,16,25–27]. The method of Siemann and Creasy [6] is often considered the benchmark assay for *trans*-resveratrol, but it requires extensive solvent extraction followed by two independent HPLC steps and UV-induced conversion of *trans*- to *cis*-resveratrol, making it unsuitable for measurement of the latter isomer. Table 4 summarizes the characteristics of these HPLC methods. The first three employ organic-phase extractions which start with volumes of wine ranging from 50 to 500 ml. Only one of these papers [7] presents data on recovery, 45% being cited. It is likely that significant losses are unavoidable whenever organic-phase extractions are employed. The method of Mattivi [15] which uses a solid-phase extraction on a C_{18} column is associated with near-100% recovery of *trans*-resveratrol [19], but in the course of the present work we experienced losses of *cis*-resveratrol ranging from 5 to 20% when using C_{18} chromatography as an initial clean-up step. This problem was resolved by direct injection, a procedure which has already been successfully employed by others [16,27].

The methods listed in Table 4 all use reversed-phase chromatography with gradient elution, and most employed more complex detection systems (diode array or fluorescence) than the single wavelength monitoring used in our method, although we did use diode-array detection for peak identification and purity checks. The sensitivities for all four constituents are superior to those of most of the methods listed in Table 4. Moreover, most start with volumes of wine ranging from 50 to 500 ml, in contrast to our method which requires direct injection of only 20 μl of wine.

The retention time of *trans*-resveratrol (the last peak of interest to elute in our system) is around 50 min, longer than most of the other methods but very much shorter than that of Roggero and Archier [16] which is able to measure polydatin as well as *trans*-resveratrol, but neither the *cis*-isomer or its glucoside. Of the

Table 4
Characteristics of previously published HPLC methods for analysis of *trans*-resveratrol

Authors [Ref.]	Column used	Elution	Detection	Retention time (min)	Sensitivity (μmol l^{-1})
Arora and Strange [25]	Spherisorb 10 ODS	Gradient[a]	Diode array	12	200
Jeandet et al. [26]	Ultrasphere ODS	Gradient[a]	307 nm	11	NS[d]
Lamuela-Raventos and Waterhouse [7]	Novapack C_{18}	Gradient[a]	Diode array	16	0.2
Mattivi [15]	Hypersil ODS	Gradient[b]	Diode array	14	0.5
Pezet et al. [27]	Lichrospher 100-RP	Gradient[c]	Fluorescence	15	0.05
			307 nm		2.0
Roggero and Archier [16]	Superspher RP-18	Gradient[c]	Diode array	120	NS
Present method	Lichrospher 100-CN	Isocratic	Absorbance	50	0.1

[a] Solvent extraction.
[b] Solid-phase extraction.
[c] Direct injection.
[d] Not stated.

remaining methods in Table 4, only one [26] measures *cis*-resveratrol simultaneously with the *trans*-isomer. The method now presented is the first which allows quantitation of both isomers and their glucosides simultaneously. The assays provide excellent linearity, stable calibration, quantitative recovery, acceptable precision, and sensitivities which are adequate for most purposes and can, if necessary, be improved by increasing the volume of sample injected (up to 100 μl is feasible). Moreover, the HPLC equipment needed is relatively standard laboratory equipment, isocratic elution is used, and *trans*-resveratrol which is commercially available is the only standard required for the calibration of all four analytes.

Methods for the analysis of *cis*-resveratrol have utilized GC [8], and GC–MS techniques [17,21], on the basis of which relatively high concentrations have been reported in commercial wines, often exceeding those of the *trans*-isomer; thus, interest in its determination is likely to increase, especially in light of a recent report that the two isomers have equivalent potency as inhibitors of protein kinases [28]. This similarity may extend to other biological actions conferring protection against coronary heart disease [10,11,29].

Polydatin has been identified as a constituent of grapes [20], but only one publication has described its presence in red wine [16]. The present report is the first to document and quantitate a glucoside of *cis*-resveratrol as well. This identification is based upon the close similarity of its UV spectrum to that of authentic *cis*-resveratrol, its sensitivity to β-glucosidase, and the fact that this enzymatic hydrolysis releases *cis*-resveratrol as validated by both HPLC and GC–MS analysis. We assume that the glucose residue is attached to the 3-position by analogy with polydatin. Linkage of a glucose moiety to the 4-position of trihydroxystilbene occurs in some plant species but has never been detected in the *Vitis* family.

Identification of polydatin, the 3-β-glucoside of *trans*-resveratrol, was achieved by the same criteria: UV spectral properties, enzymatic hydrolysis, and GC–MS analysis. In addition, the peak identified as such was shown to co-chromatograph with authentic polydatin extracted from *Polygonum cuspidatum*. Polydatin has been reported to share actions upon lipid metabolism and platelet coagulation similar to those of *trans*-resveratrol [10,11]. The biological properties of *cis*-resveratrol glucoside are unknown. However, both this and polydatin are likely to be hydrolysed by β-glucosidases present in the human intestinal tract with release of the free trihydroxystilbenes. Their assay should therefore provide a more complete characterization of the biological efficacy of individual red wines.

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