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# Method To Assay the Concentrations of Phenolic Constituents of Biological Interest in Wines

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**We describe a reversed-phase HPLC method that uses gradient elution and diode array detection to quantitate eight biologically active phenolic constituents of wine: the cis and trans isomers of resveratrol and their glucosides, catechin, epicatechin, quercetin, and rutin. ODS Hypersil served as the stationary phase; the gradient was formed by acetic acid, methanol, and water. Each analysis required an equilibration period of 10 min and a run time of 40 min for completion. Satisfactory peak resolution was achieved following direct injection of a 20-μL sample, and validation was accomplished by on-line spectral comparisons with known standards. Excellent linearity was obtained for all constituents, and the detection limits ranged from 30 μg/L (*trans*-resveratrol) to 1.5 mg/L (catechin). Recoveries approximated 100% (range 95.2–105.5%), and the method provided good precision, with coefficients of variation between 1.17 and 3.38%. All of the phenolics measured were reasonably stable in opened wines protected against sunlight for up to 1 week at room temperature or 4 °C, but most showed losses of 10–40% when stored for 6 weeks at either temperature.**

Reduced mortality from coronary heart disease (CHD) among moderate consumers of alcohol is a well-established epidemiologic phenomenon.[1-4] There is some evidence that those who regularly drink wine may have lower CHD mortality than those whose preference lies with other alcoholic beverages.[5-8] This latter possibility has provoked intense interest in constituents unique to wine that may be responsible for these putative effects. Among these, the antioxidant components of wine rank high.[9-11] Many of the latter, including catechin, epicatechin, quercetin and resveratrol (Figure 1), have been shown to protect low-density lipoproteins (LDLs) against oxidation more effectively than α-tocopherol on a molar basis.[9,12] Several of the above, as well as an extract of red wine phenolics, promote vascular relaxation through the generation of nitric oxide by the endothelium.[13] Resveratrol and quercetin inhibit human platelet aggregation in vitro and also

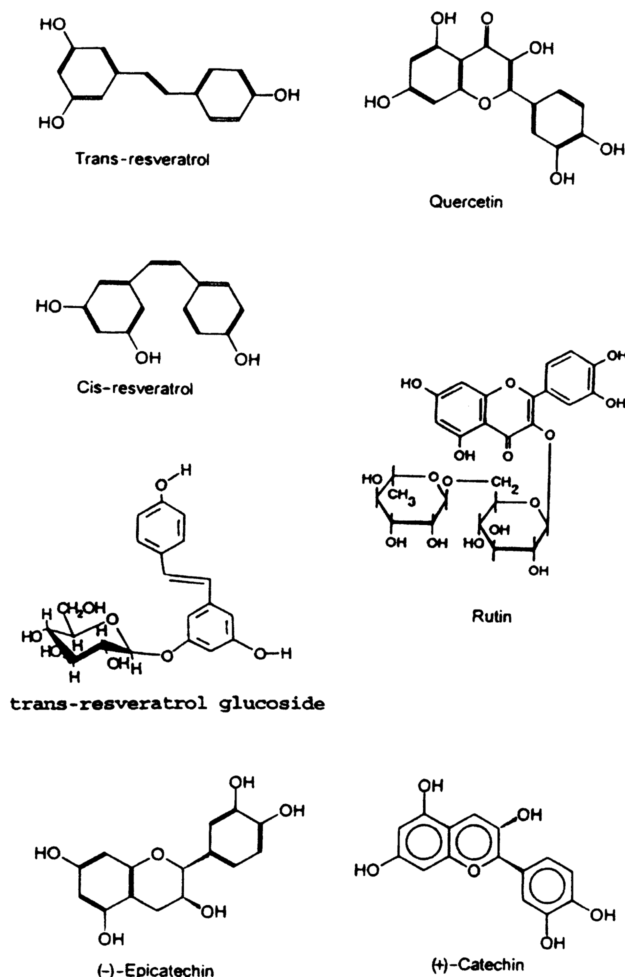


**Figure 1.** Chemical structures of phenolics measured by this method.

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(1) Rimm, E. B.; Giovannucci, E. L.; Willett, W. C.; Colditz, G. A.; Ascherio, A., Rosner, B. Stampfer, M. S. *Lancet* **1991**, *338*, 464–468.
(2) Klatsky, A. L.; Armstrong, M. A.; Friedman, G. D. *Ann. Intern. Med.* **1992**, 117, 646–654.
(3) Doll, R.; Peto, R.; Hall, E.; Wheatley, K.; Gray, R. *Br. Med. J.* **1994**, *309*, 911–918.
(4) Goldberg, D. M.; Hahn, S. E.; Parkes, J. G. *Clin. Chim. Acta* **1995**, *237*, 155–187.
(5) St. Leger, A. S.; Cochrane, A. L.; Moore, F. *Lancet* **1979**, *1*, 1017–1020.
(6) Renaud, S.; De Lorgeril, M. *Lancet* **1992**, *339*, 1523–1526.
(7) Goldberg, D. M. *Clin. Chem.* **1995**, *41*, 14–16.
(8) Groenbaek, M.; Deis, A.; Sorensen, T. I. A.; Becker, U.; Schnohr, P.; Jensen, G. *Br. Med. J.* **1995**, *310*, 1165–1169.
(9) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. *Lancet* **1993**, *341*, 454–457.
(10) Maxwell, S.; Cruickshank, A.; Thorpe, G. *Lancet* **1994**, *344*, 193–194.
(11) Whitehead, T. P.; Robinson, D.; Allaway, S.; Syms, J.; Hale, A. *Clin. Chem.* **1995**, *41*, 32–35.
(12) Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. *Lancet* **1993**, *341*, 1103–1104.



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modulate eicosanoid synthesis toward a pattern likely to be protective against CHD.[14] Red wine phenolics protect rats against the rebound hypercoagulability that follows excessive alcohol consumption.[15,16] They have also been shown to protect rats against the unfavorable lipid profiles that develop subsequent to feeding diets high in fat and cholesterol,[17] with resveratrol, quercetin, and morin perhaps the most significant in this regard.[18,19] Finally, quercetin and its glucoside rutin have potential anti-cancer effects that may provide a further dimension to the health-promoting properties of wine consumption.[20-22]

Many recent papers, including our own, have described methods to assay the content in wines of the cis and trans isomers of resveratrol as well as their corresponding glucosides, all of which are believed to share in greater or lesser degree the biological properties that have been well established for *trans*-resveratrol.[23-34] Techniques to measure other individual wine phenolics including quercetin, rutin, and the catechins, have also been reported.[35-41] These methods have generally been developed to analyze specific classes of compounds in wine and grapes, e.g., procyanidins and anthocyanins, and have been applied to the examination of various aspects of fermentation chemistry. As such, they are capable of measuring some of the components of major biological interest described above. However, it is now becoming apparent that an index of the potential health benefits of individual wines requires knowledge of the concentrations of all constituents, including those already mentioned, which may have biological properties favoring protection against the major causes of adult mortality, such as CHD and cancer. A number of more general methods based on high-performance liquid chromatography (HPLC) have been developed[42-44] that allow quantitation of up to 20 wine phenolics simultaneously on a single sample, but these have not been adequately described or characterized as to their analytical performance; neither have they been applied to a sufficiently representative number or range of wines to establish their suitability and robustness for routine use; nor do they necessarily include all of the major compounds of biological interest. We now describe a HPLC technique based on direct sample injection and diode array detection that meets all of these criteria.

## MATERIALS AND METHODS

**Wines.** Commercial wines, usually in 750-mL bottles, were opened, and 10-mL aliquots were withdrawn for storage at 4 °C in a glass vial filled to completion and protected by foil against sunlight. Analyses were completed within a 3–5-day period.

**Standards.** The following were purchased from Sigma (St. Louis, MO) and used for calibration: catechin (Catalog No. C1251), epicatechin (Catalog No. E1753), *trans*-resveratrol (Catalog No. R5010), rutin (Catalog No. R5143), and quercetin (Catalog No. Q0125). *cis*-Resveratrol was prepared from the trans isomer by UV irradiation.[34] *trans*-Polydatin was isolated from the dried roots of *Polygonum cuspidatum*, and a portion was converted to the cis isomer by UV irradiation.[34] All standards were dissolved in methanol at a range of concentrations described under Linearity in the next section.

**Instrumentation.** An ODS Hypersil 5-μm column, 250 mm × 4 mm i.d., was used as the stationary phase and was preceded by a guard column of LiChrospher 100 RP-18, 5 μm, 4 mm × 4 mm. Both were purchased from Hewlett Packard (Mississauga, ON, Canada). The chromatography equipment, all from Hewlett Packard, comprised the Series 1050 automatic sample injector, solvent degasser, quaternary pump, and diode array detector coupled to the HP Chem-Station utilizing the manufacturer's 2.05 software package.

**Procedure.** Samples of 20 μL of wine or calibration standard were directly injected onto the column and eluted with a gradient comprising acetic acid (pump A), methanol (pump B), and water (pump C). Zero-time conditions were 5% A, 15% B, 80% C at a flow rate of 0.4 mL/min. After 5 min, the pumps were adjusted to 5% A, 20% B, 75% C at a flow rate of 0.5 mL/min, and at 30 min to 5% A, 45% B, 50% C at 0.5 mL/min until termination of the run at 40 min. This was followed by a 10-min equilibrium period with the zero-time solvent mixture prior to injection of the next sample. Detection was routinely accomplished by monitoring the absorbance signals at 265, 280, 306, 317, and 369 nm with a bandwidth of 5 nm. Match and purity checks were performed for all peaks of interest as described in the next section. A standard was injected after every five samples, and after every 16 samples the column was washed for 2 h with water at 1 mL/min, followed by 2 h with methanol at the same rate. The column could be used for at least 200 assays; the earliest manifestation of deterioration


(13) Fitzgerald, D. F.; Hirschfield, S. L.; Coffey, R. G. *Am. J. Physiol.* **1993**, *265*, H774–H778.
(14) Pace-Asciak, C. R.; Hahn, S.; Diamandis, E. P.; Soleas, G.; Goldberg, D. M. *Clin. Chim. Acta* **1995**, *235*, 207–219.
(15) Ruf, J. C.; Berger, J.-L.; Renaud, S. *Arterioscler. Thromb. Vasc. Biol.* **1995**, *15*, 140–144.
(16) Renaud, S.; Ruf, J. C. *Clin. Chim. Acta*, in press.
(17) Tebib, K.; Bitri, L.; Besancon, P.; Rouanet, J.-M. *Food Chem.* **1994**, *49*, 403–406.
(18) Arichi, H.; Kimura, Y.; Okuda, H.; Baba, K.; Kozawa, M.; Arichi, S. *Chem. Pharm. Bull.* **1982**, *30*, 1766–1779.
(19) Yugarani, T.; Teh, B. K. H.; The, M.; Das, N. P. *Lipids* **1992**, *27*, 181–186.
(20) Deschner, E. E.; Ruperto, J.; Wong, G.; Newmark, H. L. *Carcinogenesis* **1991**, *12*, 1193–1196.
(21) Yoshida, M.; Yamamoto, M.; Nikaida, T. *Cancer Res.* **1992**, *52*, 6676–6681.
(22) Avila, M. A.; Velasco, J. A.; Cansado, J.; Notario, V. *Cancer Res.* **1994**, *54*, 2424–2428.
(23) Siemann, E. H.; Creasey, L. L. *Am. J. Enol. Vitic.* **1992**, *43*, 49–52.
(24) Jeandet, P.; Bessis, R.; Maume, B.; Sbaghi, M. *J. Wine Res.* **1993**, *4*, 79–85.
(25) Lamuela-Raventos, R. M.; Waterhouse, A. L. *J. Agric. Food Chem.* **1993**, *41*, 521–523.
(26) Mattivi, F. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 522–525.
(27) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E. P.; Karumanchiri, A.; Soleas, G.; Waterhouse, A. L. *Anal. Chem.* **1994**, *66*, 3959–3963.
(28) Pezet, R.; Pont, V.; Cuenat, P. *J. Chromatogr.* **1994**, *663*, 191–197.
(29) Roggero, J. P.; Archier, P. *Sci. Aliments* **1994**, *14*, 99–107.
(30) Lamuela-Raventos, R. M.; Romero-Perez, A. I.; Waterhouse, A. L.; De La Torre-Boronat, M. C. *J. Agric. Food Chem.* **1995**, *43*, 281–283.
(31) Goldberg, D. M.; Karumanchiri, A.; Ng, E.; Yan, J.; Diamandis, E. P.; Soleas, G. J. *J. Agric. Food Chem.* **1995**, *43*, 1245–1250.
(32) Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P. *Am. J. Enol. Vitic.*, in press.
(33) Soleas, G. J.; Goldberg, D. M.; Diamandis, E. P.; Karumanchiri, A.; Yan, J.; Ng, E. *Am. J. Enol. Vitic.* **1995**, *46*, 346–352
(34) Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E. P.; Soleas, G. J. *J. Chromatogr.* **1995**, *708*, 95–89
(35) Nagel, C. W.; Wulf, L. W. *Am. J. Enol. Vitic.* **1979**, *30*, 111–116.
(36) Singleton, V. L.; Trousdale, E. *Am. J. Enol. Vitic.* **1983**, *34*, 27–34.
(37) Salagoity-Auguste, M. H.; Bertrand, A. *J. Sci. Food Agric.* **1984**, *35*, 1241–1247.
(38) Jaworski, A. W.; Lee, C. Y. *J. Agric. Food Chem.* **1987**, *35*, 257–259.
(39) Da Silva, J. M. R. *J. Sci. Food Agric.* **1990**, *53*, 85–92.
(40) Spanos, G. A.; Wrolstad, R. E. *J. Agric. Food Chem.* **1990**, *38*, 1565–1571.
(41) Oszmianski, J.; Lee, C. Y. *Am. J. Enol. Vitic.* **1990**, *41*, 204–206.
(42) Roggero, J.-P.; Coen, S.; Archier, P. *J. Liq. Chromatogr.* **1990**, *13*, 2593–2603.
(43) Roggero, J.-P.; Archier, P.; Coen, S. *J. Liq. Chromatogr.* **1991**, *14*, 533–538.
(44) Lamuela-Raventos, R. M.; Waterhouse, A. L. *Am. J. Enol. Vitic.* **1994**, *45*, 1–5.

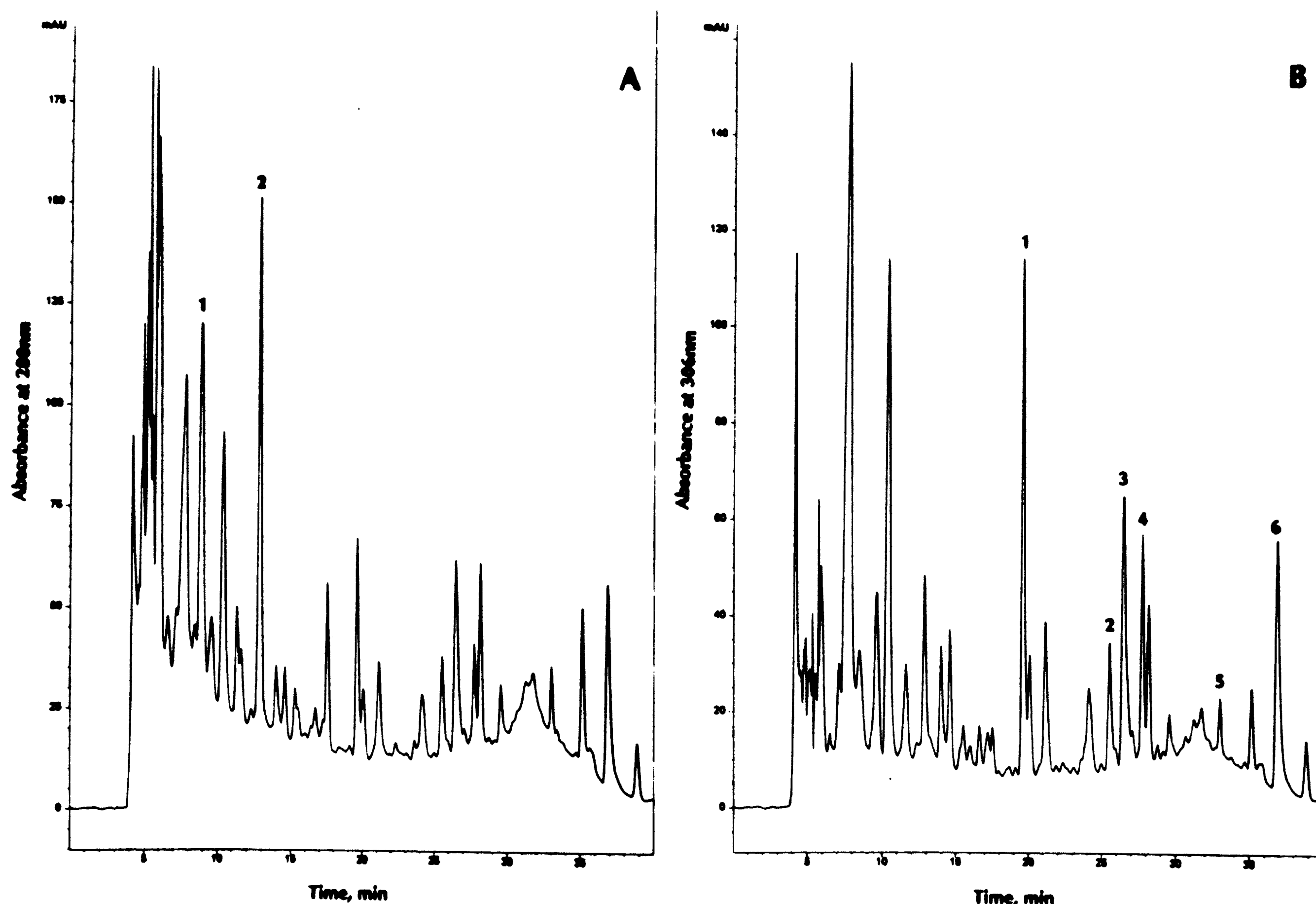


**Figure 2.** Chromatogram of wine showing the peaks for all eight phenolics measured by this method. (A) Absorbance monitored at 280 nm. Peak 1, catechin; peak 2, epicatechin. (B) Absorbance monitored at 306 nm. Peak 1, *trans*-polydatin; peak 2, rutin; peak 3, *trans*-resveratrol; peak 4, *cis*-polydatin; peak 5, *cis*-resveratrol; peak 6, quercetin.

was inadequate resolution of the *cis*-polydatin and *trans*-resveratrol peaks.

## RESULTS

**Chromatographic Resolution.** Figure 2 demonstrates the satisfactory resolution accomplished for the major peaks of interest when the eluate was monitored at wavelengths of 280 and 306 nm. Although some peaks show minor shoulders, the software can apply adequate corrections during integration, which can also be done manually for sharper definition of the true peaks. Match factor spectral analysis of each peak assigned a value between 0 and 1000 for concordance between the spectrum of the peak and that of the pure standard of the compound which it was assumed to be. According to the manufacturer, values above 990 indicate near-identity, and those below 900 suggest that the spectra are different. We used a value of 950 as our criterion for acceptability on the basis that, in analyses of 100 wines, 95% of all peaks gave a higher match factor. Examples of peaks with acceptable (99.9%) and unacceptable (82.5%) match factors are illustrated in Figure 3 parts A and B, respectively. Purity checks were also performed at the inflexion points and apex of each peak, and the peak purity plot comprising the three spectra was drawn in a normalized and overlayed mode. By the same criterion as employed to define the acceptable limit for match factor, purity factors >950 led us to exclude hidden impurities and to consider the peak to be consistent with the presence of a single component. Examples of satisfactory (99.9%) and unsatisfactory (72.6%) purity factors are provided in Figure 4 parts A and B, respectively.

**Table 1. Linearity of Assays for Eight Phenolic Constituents of Wine Assessed by Regression Analysis ($y$ = Area Count; $x$ = Concentration in mg/L)**

| constituent | slope | intercept | SE | $P$[a] | $r$[b] | range[c] |
|---|---|---|---|---|---|---|
| catechin | 0.9968 | 33.7 | 42.3 | 0.48 | 0.999 | 10–200 |
| epicatechin | 0.9994 | −33.1 | 20.5 | 0.18 | 1.000 | 10–100 |
| rutin | 0.9995 | −43.4 | 25.6 | 0.16 | 1.000 | 5–50 |
| *trans*-resveratrol | 0.9997 | 42.7 | 14.7 | 0.04 | 1.000 | 0.4–8.5 |
| *cis*-resveratrol | 0.9998 | 23.1 | 7.6 | 0.04 | 1.000 | 0.6–13.2 |
| quercetin | 0.9985 | −241.7 | 92.4 | 0.06 | 0.999 | 5–50 |
| *trans*-polydatin | 0.9689 | −65.9 | 127.3 | 0.64 | 0.984 | 0.9–6.8 |
| *cis*-polydatin | 0.9624 | 59.4 | 132.6 | 0.68 | 0.980 | 1.4–10.2 |

[a] Probability that intercept is significantly different from zero. [b] Correlation coefficient. [c] Lowest and highest concentrations in mg/L.

**Linearity.** Data for each constituent were pooled from at least three experiments in which the constituent was analyzed over a range of 6–10 concentrations individually, in a mixture of all eight dissolved in methanol, and added to a white wine matrix. Multiple regression analyses were performed using the formula $y = ax^3 + bx^2 + cx + d$. From Table 1, it may be seen that the slope of the calibration curve was almost perfectly linear in all instances, and that for all but two, the correlation coefficient differed from unity (if at all) only in the fourth decimal place. The range of concentrations covered those seen in >95% of all red wines, although many white wines have lower concentrations than the lowest used for several constituents. The ranges of resveratrol isomers and glucosides could not be strictly predetermined

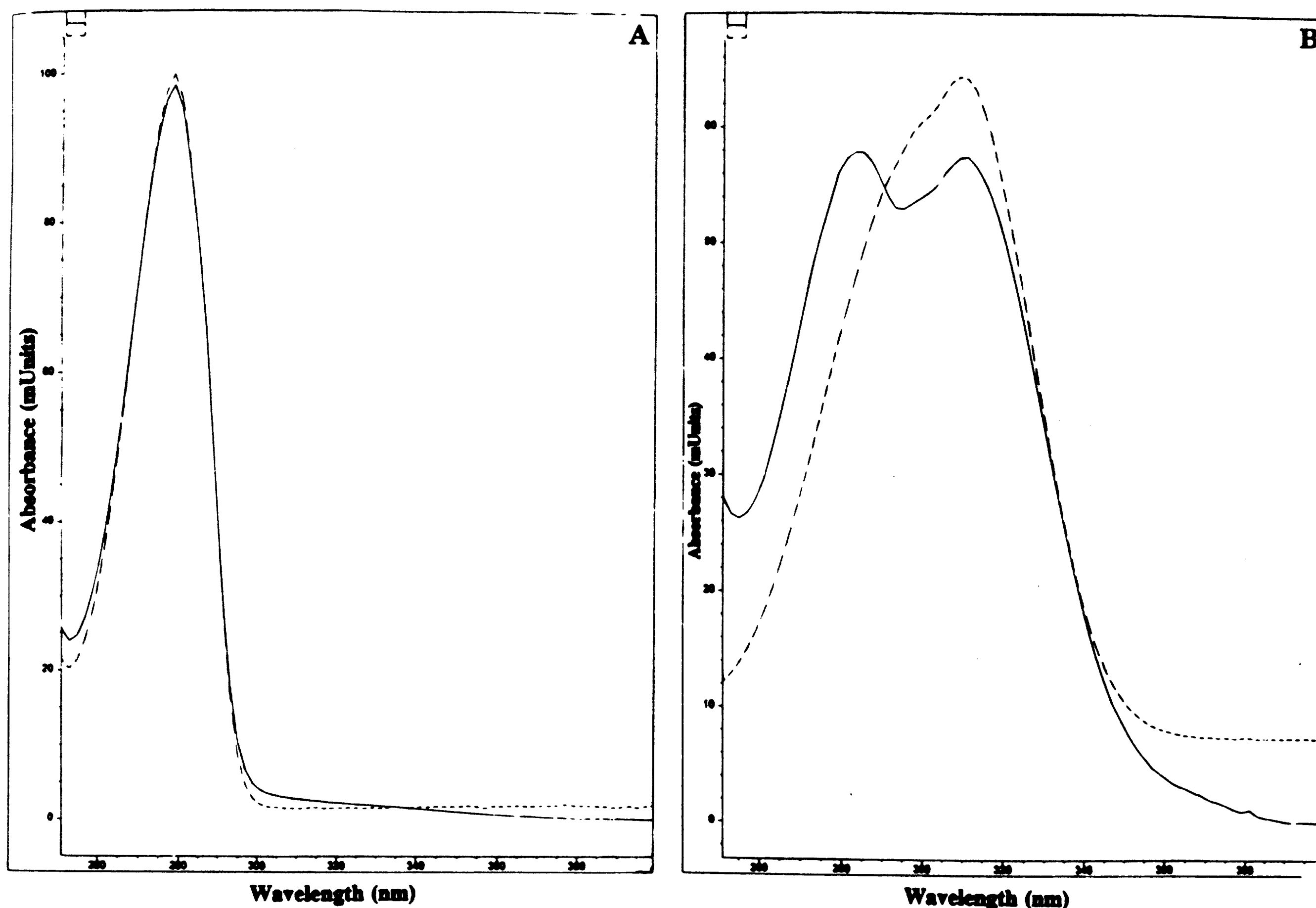


**Figure 3.** Match factor spectral analysis of two peaks eluting at retention time of epicatechin. The solid line is the spectrum of an actual column effluent, and the broken line is the spectrum of pure epicatechin retrieved from a library. (A) Near-identity of spectra with match factor of 99.9%. (B) Unacceptable identity with match factor of 82.5%. The spectrum of the effluent is closer to that of *p*-coumaric acid than to that of epicatechin.

because they were obtained by UV irradiation of pure solutions of the *trans* isomers, but all values were subsequently assigned on the basis of analysis by our previously published GC/MS[27,31] and normal-phase HPLC[34] methods and were confirmed by the fact that the sum of the isomers and glucosides after irradiation was equal to the initial concentration of the trans isomer.

Only with *cis*- and *trans*-resveratrol did the intercept (positive) differ significantly from zero ($P < 0.04$ for each). This is consistent with the notion of background or baseline interference or lack of complete resolution in some matrixes. Numerically, this was <2% of the average concentration of these compounds in red wine and was insufficient to adversely affect other analytical variables such as sensitivity, recovery, and precision. Moreover, the match factor and purity checks eliminated any data that did not meet stringent criteria and presumably protected against errors due to such interference, especially at low concentrations.

**Recovery.** This was evaluated for each constituent by adding two concentrations to red (wine A), rosé (wine B), and white (wine C) commercial wines and performing quadruplicate assays before and after each addition. Excellent recovery was obtained, which on average (Table 2) ranged from 95.2 ± 5.5% for catechin to 105.5 ± 4.3% for *trans*-resveratrol. There was no consistent difference in recovery from any of the three wine matrixes.

**Precision.** Ten replicate analyses were performed on four wines for each of the eight phenolics, selected from a number previously assayed so as to cover a reasonable range for each constituent. Rutin was not measurable in two and quercetin in one because of match or purity factors that did not meet the defined criteria. The mean coefficients of variation (CVs) indicated in Table 3 ranged from 1.17 to 3.38%, and the highest single value was for *cis*-resveratrol (5.7%) at a concentration of 0.79 mg/L. This represents excellent precision for all constituents throughout the range tested.

**Detection Limit.** Basing this on the lowest value that could be distinguished from zero (baseline) at $P < 0.001$ with a CV of ≤10% and acceptable spectral criteria (match and purity factors), the lowest measurable values and best wavelengths were as follows: catechin, 1.5 mg/L (280 nm); epicatechin, 1.2 mg/L (280 nm); *cis*-polydatin, 75 μg/L (285 nm); *trans*-polydatin, 48 μg/L (306 nm); *cis*-resveratrol, 135 μg/L (285 nm); *trans*-resveratrol, 30 μg/L (306 nm); rutin, 0.8 mg/L (265 nm); and quercetin, 0.4 mg/L (369 nm).

**Stability.** Ten wines were analyzed immediately and divided into two aliquots, which were stoppered in dark glass vials, covered with aluminum foil, and kept in the dark for up to 6 weeks at room temperature and 4 °C. Replicate analyses were performed on both aliquots at 48 h, 1 week, and 6 weeks. Table 4 gives the mean percentage change for each constituent; because of considerable skewing of the data due to exaggerated changes in certain constituents in some samples, the observed range of values as a percentage of the zero-time analysis is also given. The paired *t*-test was used to evaluate the significance of these changes. The quercetin and rutin peaks met the spectral criteria in only six and four samples, respectively, and the latter were not included in the

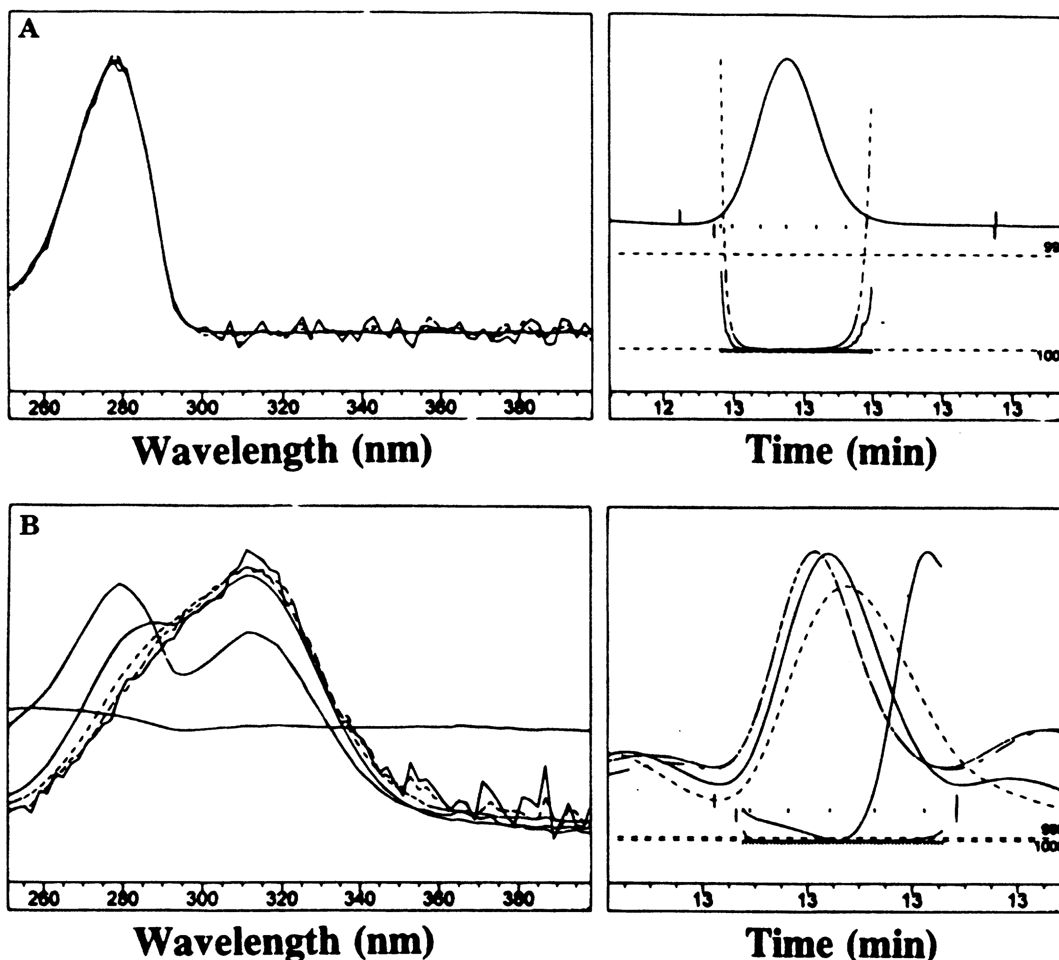


**Figure 4.** Purity check spectral analysis of peak eluting at retention time of epicatechin. (A) Purity confirmed (99.9%). (B) Purity not confirmed (72.6%).

**Table 2. Recovery (%) of Eight Phenolic Constituents Added to Each of Three Wines at High Concentration (mg/L, Indicated in Parentheses beside Each Constituent) and Low Concentration (Half of High Concentration)**[a]

| constituent | wine A high | wine A low | wine B high | wine B low | wine C high | wine C low | overall[b] mean ± SD |
|---|---|---|---|---|---|---|---|
| catechin (80) | 82.9 | 92.2 | 103.9 | 106.3 | 96.2 | 99.3 | 95.2 ± 5.5 |
| epicatechin (80) | 116.6 | 101.5 | 94.4 | 99.1 | 103.7 | 104.7 | 103.5 ± 4.6 |
| *cis*-polydatin (7.32) | 101.1 | 94.3 | 107.4 | 107.5 | 91.5 | 97.4 | 99.8 ± 4.6 |
| *trans*-polydatin (8.66) | 107.4 | 99.3 | 106.0 | 106.4 | 97.7 | 101.4 | 103.1 ± 2.3 |
| *cis*-resveratrol (3.14) | 82.2 | 92.4 | 98.7 | 101.3 | 105.7 | 104.8 | 97.5 ± 4.5 |
| *trans*-resveratrol (3.32) | 112.1 | 98.2 | 115.1 | 114.1 | 95.2 | 97.9 | 105.5 ± 4.3 |
| rutin (36) | 116.3 | 107.7 | 85.8 | 85.2 | 102.1 | 102.1 | 99.8 ± 8.5 |
| quercetin (38) | 105.4 | 102.6 | 102.1 | 89.9 | 99.1 | 88.9 | 100.0 ± 5.4 |

[a] Each wine was assayed four times before and after each addition, and average of results is presented [b] Obtained by pooling all recovery data, i.e., $n = 24$.

statistical analysis. With the exception of epicatechin at room temperature which showed a slight (1.72%) but significant ($P < 0.05$) reduction, all constituents were stable over 48 h. At 1 week, minor losses of 2.95% (*trans*-resveratrol), 4.3% (epicatechin), 7.21% (catechin), and 10.62 (*cis*-polydatin) were recorded at room temperature ($P < 0.05$), but at 4 °C, only a small reduction in epicatechin concentration of 2.9% was significant ($P < 0.05$). After 6 weeks, major losses occurred in all constituents at room temperature ranging from 13.3% (epicatechin) to 43.5% (catechin). At 4 °C, these losses were much reduced, ranging from 4.1% (epicatechin) to 25.3% (*cis*-polydatin), but all were highly significant. For practical purposes, it seems that reasonably reliable results may be expected for most wines protected from light when analysis is delayed for up to 48 h or for up to 1 week at 4 °C. Longer storage is not advisable once the bottle has been opened and exposed to air.

**Application.** This method has been in use for the analysis of >250 wines, and we intend to complete a survey of at least 1000 commercial wines in an effort to define the phenolic content of those from the major regions and countries of production. To demonstrate the scope of the method and to provide an indication of the range of concentrations that may be expected for each constituent, Table 5 presents the results of analyses of two representative wines from each of nine different countries or

**Table 3. Precision of HPLC Assays for Eight Wine Phenolics[a]**

| constituent | mean (mg/L) | SD (mg/L) | CV (%) | overall[b] mean CV (%) |
|---|---|---|---|---|
| catechin | 148 | 0.8 | 0.5 | |
| | 48 | 1.1 | 2.3 | |
| | 34 | 0.6 | 1.8 | |
| | 296 | 1.2 | 0.4 | 1.25 |
| epicatechin | 49 | 0.5 | 1.0 | |
| | 20 | 0.5 | 2.5 | |
| | 108 | 0.5 | 0.5 | |
| | 71 | 0.5 | 0.7 | 1.17 |
| *cis*-polydatin | 1.7 | 0.03 | 1.8 | |
| | 1.1 | 0.02 | 1.8 | |
| | 1.5 | 0.03 | 2.0 | |
| | 0.95 | 0.036 | 3.8 | 2.35 |
| *trans*-polydatin | 4.1 | 0.10 | 2.4 | |
| | 4.4 | 0.12 | 2.7 | |
| | 1.98 | 0.02 | 1.0 | |
| | 1.32 | 0.03 | 2.3 | 2.10 |
| *cis*-resveratrol | 4.7 | 0.07 | 1.5 | |
| | 2.9 | 0.10 | 3.4 | |
| | 0.79 | 0.045 | 5.7 | |
| | 2.8 | 0.08 | 2.9 | 3.38 |
| *trans*-resveratrol | 4.3 | 0.06 | 1.4 | |
| | 1.72 | 0.08 | 4.7 | |
| | 1.66 | 0.02 | 1.2 | |
| | 1.85 | 0.01 | 0.5 | 1.95 |
| quercetin | 9.5 | 0.14 | 1.5 | |
| | 13.6 | 0.38 | 2.8 | |
| | 20.6 | 0.42 | 2.4 | 2.23 |
| rutin | 9.2 | 0.35 | 3.8 | |
| | 11.6 | 0.13 | 1.1 | 2.45 |

[a] Each value is derived from 10 replicate analyses on up to 4 wines of varying concentration for each constituent. [b] Obtained by averaging the CVs for all wine samples, i.e., $n = 4$ for all except quercetin ($n = 3$) and rutin ($n = 2$).

**Table 4. Stability of Wine Phenolics Based on Replicate Analyses of the Same 10 Wines Stored at Two Temperatures over Three Time Intervals[a]**

| | 48 h | | 1 week | | 6 weeks | |
|---|---|---|---|---|---|---|
| constituent | rt[b] | 4 °C | rt[b] | 4 °C | rt[b] | 4 °C |
| catechin | −1.75 (102.2−94.9) | −1.05 (105.3−92.2) | −7.21 (98.2−88.0) | −3.79 (102.5−86.0) | −43.5 (95.0−45.5) | −8.66 (98.1−80.3) |
| epicatechin | −1.72 (98.7−97.4) | −0.11 (102.0−98.8) | −4.3 (99.4−92.8) | −2.9 (98.4−94.6) | −13.3 (93.9−80.7) | −4.1 (98.7−91.8) |
| *cis*-polydatin | −2.51 (110.4−86.5) | −3.1 (104.4−87.6) | −10.62 (102.7−80.4) | −4.9 (105.5−84.9) | −31.93 (89.4−51.2) | −25.3 (94.6−55.4) |
| *cis*-resveratrol | +4.74 (111.4−89.8) | +5.56 (115.4−95.9) | −3.55 (110.7−80.7) | +2.01 (116.3−81.2) | −17.49 (92.2−70.4) | −11.66 (98.6−72.3) |
| *trans*-polydatin | +1.22 (104.5−96.0) | +2.14 (106.9−97.2) | −1.14 (107.7−92.7) | 0.45 (106.3−89.7) | −19.04 (95.6−61.8) | −7.86 (102.4−81.1) |
| *trans*-resveratrol | −0.80 (104.3−93.4) | +0.55 (104.7−96.5) | −2.95 (99.8−95.0) | −1.24 (106.5−90.1) | −27.55 (90.0−60.2) | −15.61 (96.3−71.4) |
| rutin[c] | −1.91 (100.4−92.2) | −2.2 (100.5−95.4) | −5.3 (99.6−81.3) | −4.8 (100.3−83.8) | −14.4 (96.4−75.5) | −11.2 (95.9−78.8) |
| quercetin[d] | +1.11 (103.2−95.6) | +1.46 (105.9−97.8) | −4.42 (102.8−92.6) | −1.94 (103.4−90.2) | −42.77 (73.7−41.1) | −16.47 (95.3−70.8) |

[a] The open figures represent the average percentage change under each condition, and the figures in parentheses represent the range of values for each time point and storage condition, expressed as a percent of the zero time baseline. [b] Room temperature. [c] Based on four samples. [d] Based on six samples.

**Table 5. Phenolic Composition of Representative Commercial Wines (mg/L)**

| wine | catechin | epicatechin | *trans*-polydatin | *trans*-resveratrol | *cis*-polydatin | *cis*-resveratrol | rutin | quercetin |
|---|---|---|---|---|---|---|---|---|
| 1991 Chianti (Italy) | 34.1 | 22.9 | 2.20 | 1.28 | 1.00 | 0.70 | | 7.39 |
| 1990 Chianti | 39.6 | 22.7 | 1.15 | 1.17 | 1.26 | 0.63 | | 8.67 |
| 1990 Chateauneuf (France) | 50.3 | 23.2 | 3.59 | 4.68 | 2.03 | 1.94 | | 2.68 |
| 1991 Chateauneuf | 55.2 | 23.5 | 2.66 | 3.82 | 1.32 | 1.12 | | 0.29 |
| 1990 Medoc (France) | 48.9 | 38.1 | 0.72 | 1.72 | 0.49 | 1.59 | | 0.88 |
| 1990 Medoc | 58.2 | 30.6 | 0.15 | 2.32 | 0.18 | 1.29 | | 0.60 |
| 1989 Burgundy (France) | 127.0 | 38.4 | 2.34 | 2.27 | 0.98 | 1.44 | | 3.14 |
| 1989 Burgundy | 136.0 | 50.7 | 2.22 | 1.17 | 1.12 | 0.98 | | 2.20 |
| 1990 California Cabernet Sauvignon | 33.5 | 21.4 | 0.24 | 0.30 | 0.10 | 0.30 | 14.6 | 3.15 |
| 1991 California Cabernet Sauvignon | 44.4 | 23.1 | 1.86 | 2.23 | 1.52 | 1.11 | 2.42 | 1.17 |
| 1993 Australian Shiraz | 44.2 | 34.9 | 1.45 | 2.62 | 0.19 | 1.95 | | 6.53 |
| 1991 Australian Shiraz | 33.9 | 33.7 | 0.72 | 2.26 | 0.32 | 1.16 | | 9.89 |
| 1991 Oregon Pinot Noir | 122 | 41.6 | 0.84 | 0.50 | 1.74 | 1.06 | | 1.56 |
| 1991 Oregon Pinot Noir | 119 | 38.6 | 0.83 | 0.45 | 2.05 | 0.94 | | 2.70 |
| 1993 Beaujolais (France) | 31.5 | 24.2 | 1.72 | 2.29 | 2.64 | 2.31 | | 5.03 |
| 1993 Beaujolais | 30.7 | 64.4 | 1.76 | 2.69 | 2.90 | 2.95 | | 5.15 |
| 1988 Barolo (France) | 23.6 | 16.7 | 0.98 | 0.31 | 1.17 | 0.50 | | 4.55 |
| 1989 Barolo | 59.0 | 34.3 | 1.46 | 1.04 | 0.79 | 0.44 | | 9.41 |

regions. These data do not merit detailed description beyond pointing out a few salient observations: (1) the concentrations of catechin and epicatechin are at least an order of magnitude higher than those of the other phenolics measured; (2) whereas the concentrations of the resveratrol isomers and glucosides span a rather narrow range, those of quercetin are much wider, and rutin was measurable in only two of the 18 wines; and (3) a number of trends appear worthy of investigation, including the high catechin content of Burgundy and Oregon wine and the high quercetin content of Italian and Australian wine, although these features are as likely to be due to the grape cultivar as to environmental factors.

## DISCUSSION

In this paper, we have described a method for the analysis of eight phenolic constituents of wine by HPLC of a 20-μL sample which is directly introduced without the need for prior preparatory procedures. The analysis time of 40 min allows a relatively fast throughput, and with automatic sample injection, 20 samples can be run every 24 h, including time for standardization and column-washing. Linearity, recovery, precision, and sensitivity were highly satisfactory. Given the good peak resolution (Figure 2), many more compounds can be analyzed than those now reported. Since commencing this study, we have been able to measure several additional compounds, including morin, salicylic acid, and *p*-coumaric acid, although only the latter has been detected by us in commercial wines with any regularity. In theory, it should be possible to assay for any wine phenolic constituent for which a standard is available, which absorbs over the range of wavelengths utilized, and which provides a reasonably discrete peak. A number of such peaks, hitherto unidentified, are present in the chromatograms illustrated in Figure 2 and are much more prominent at other wavelengths. Several may turn out to be among the constituents reported by other authors as measurable in red wine extracts by various HPLC methods.

Although all of these phenolics have been measured in wine previously, this is the first report documenting a method to measure the eight compounds of interest simultaneously and by a technique that requires no sample preparation. The characteristics of the method have also been thoroughly delineated for each constituent and compare favorably with those described by others, although the requisite details are frequently not provided. Nagel and Wulf[35] gave no information for their method, which included the assay of catechin and epicatechin. Although some authors have made general comments about linearity, only one presented relevant data showing linearity for catechin and epicatechin to 30 mg/L and for quercetin to 20 mg/L.[37] Recoveries have been described for catechin and epicatechin of 91–92%,[38] 98–101%,[39] and 79–92%.[40] Very variable precision has been reported for catechin and epicatechin: 1.2–3.5%,[36] 15–27%,[37] 5.8%,[38] 2.8–3.2%,[39] and 10%.[41] For quercetin, precision values of 18%[37] and 5%[41] have been recorded, and for rutin 9.9%[41] has been recorded. A clearly defined detection limit of 1.2 mg/L has been documented for catechin and epicatechin by only one author.[39]

The stability of wine phenolics once the bottle has been opened has not been evaluated previously, except for *trans*-resveratrol. In contrast to our previous results, which showed this compound to be stable at 4 °C for up to 6 weeks,[27] a loss of 15.6% was noted in the present study. It is possible that part of this loss is due to oxidative dimerization, which is known to occur with resveratrol.[45] If so, it would not coelute with free resveratrol on HPLC, but its fragmentation at the high temperatures employed in the direct injection GC/MS assay used in our earlier investigation[27] could well generate a mass-ion pattern augmenting the resveratrol signal and masking the loss. The chemical changes that underlie the reduced concentrations of wine phenolics during storage merit independent investigation in their own right.

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