

A Validated HPLC Method for the Quantitative Analysis of *Trans*-Resveratrol and *Trans*-Piceid in Hungarian Wines

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Abstract

A new, simple procedure for the determination of phenolic components of wines, such as resveratrol and piceid isomers, has been elaborated and validated. A set of 70 red wines and 3 white wines from two wineries (Polgar Winery and Bock Winery, Villany, Hungary) are analyzed by reversed-phase high-performance liquid chromatography. The samples are injected without pretreatment and UV-vis and mass spectrometric (MS) detection has been applied. The detection limit for *trans*-resveratrol and for *trans*-piceid is found to be 0.9 and 0.6 pmol for the UV-vis detection method and 0.3 and 0.2 pmol for the MS detection method. *Trans*-resveratrol and *trans*-piceid are found in red wines from 0.1 to 14.3 mg/L and from 3.8 to 16.4 mg/L concentrations, respectively.

Introduction

Resveratrol and piceid (Figure 1) isomers are natural phenolic components in wines and in many other families of plants (1). Resveratrol is a phytoalexin synthesized in the plant against

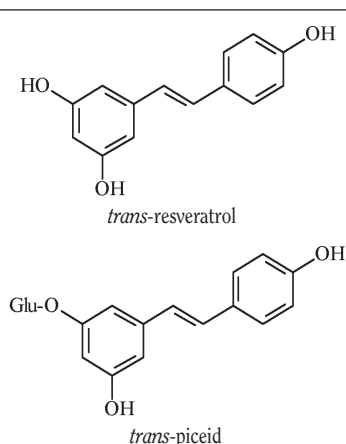


Figure 1. The chemical formulae of *trans*-resveratrol and *trans*-piceid.

stress, such as attacks by pathogens, UV radiation, mechanical injury, or heavy metal pollution. *Trans*-resveratrol (3,4',5-trihydroxy-*trans*-stilbene) has many beneficial effects for human health (2). It is an antioxidant, which inhibits low density lipoprotein oxidation (3). It probably has an anticarcinogen effect (4–6) and is important in the prevention of cardiovascular diseases (7,8). It protects against cerebral ischemic injury (9) and activates sirtuin 1, causing fat reduction in white adipocytes (10,11). Some studies focused on polyphenolic composition of wines throughout the wine-making regions of the world (12,13). In recent investigations, the preferred analytical methods are high-performance liquid chromatography (HPLC) with diode array UV-vis detection, mass spectrometric (MS) detection, and capillary electrophoresis (14–17).

Experimental

Chemicals and reagents

The *trans*-resveratrol standard (99%) was purchased from Sigma-Aldrich (Budapest, Hungary), the *trans*-piceid standard from Herbestandard Inc. (Chesterfield, MO), acetic acid (96%) from Riedel-de Haën GmbH & Co. (Seelze, Germany), and methanol (HPLC-grade) from Scharlau Chemie S.A. (Barcelona, Spain). All other chemicals were of analytical grade. Freshly bidistilled water was used for the preparation of the aqueous solutions. The wine samples were gifts of the Polgar and Bock Wineries (Villany, Hungary).

Standard solutions and sample preparation

The standards were dissolved in a small portion of ethanol and filled up with the eluent. The wine samples were directly injected without pretreatment. All standard solutions and wine samples were stored in the darkness at 5°C to avoid oxidative degradation and isomerization of the *trans*-resveratrol and *trans*-piceid to the *cis*- ones.

HPLC instrumentation and conditions

The HPLC system consisted of a Gynkotek M 580 GT pump, Rheodyne 8125 injector (20- μ L loop) (Cotati, CA), and a

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Gynkotek M 340S UV diode-array detector (Gynkotek GmbH, Germering, Germany). A column (250 × 4.6 mm) packed with 6- μm particle size C_{18} material (18) has been used for the separations. A Chromeleon data management software (Dionex Corp., Sunnyvale, CA) was used for the control of the equipment and for data evaluation. Quantization was carried out using the peak areas method. A multistep gradient method was applied using methanol–water–acetic acid (10:90:1, v/v) mixture as solvent A and methanol–water–acetic acid (90:10:1, v/v) mixture as solvent B at a flow rate of 1.5 cm^3/min . The gradient profile was 0.0–18.0 min from 0% to 40% B, 18.0–25.0 min from 40% to 100% B, and 25.0–27.0 min 100% B. Chromatographic separations were monitored at 306 nm. Chromatographic peaks were identified by comparing retentions and UV and MS spectra of the samples with those of the standard compounds. Quantitation was carried out by external standardization (Figure 2).

MS instrumentation and conditions

MS analysis was performed using a Finnigan AQA (Thermo-

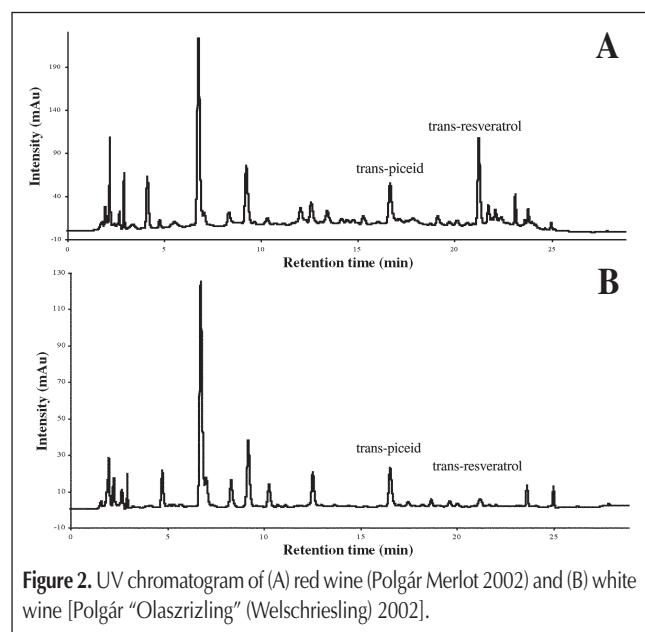


Figure 2. UV chromatogram of (A) red wine [Polgár Merlot 2002] and (B) white wine [Polgár "Olaszrizling" (Welschriesling) 2002].

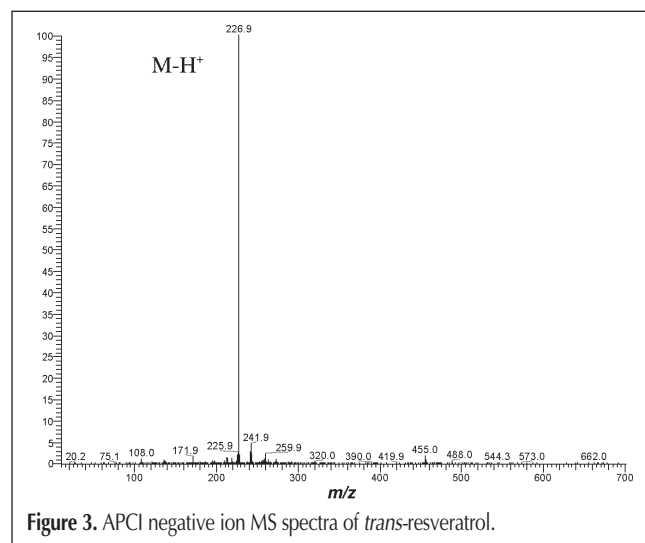


Figure 3. APCI negative ion MS spectra of *trans*-resveratrol.

Quest, San José, CA) MS equipped with both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) interfaces. Both the auxiliary and the curtain gas were nitrogen at the flow rate of 600 L/h. For LC–MS analysis, APCI

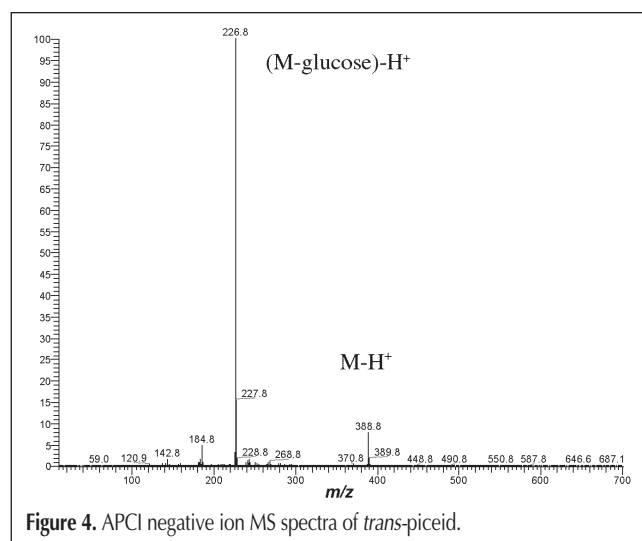


Figure 4. APCI negative ion MS spectra of *trans*-piceid.

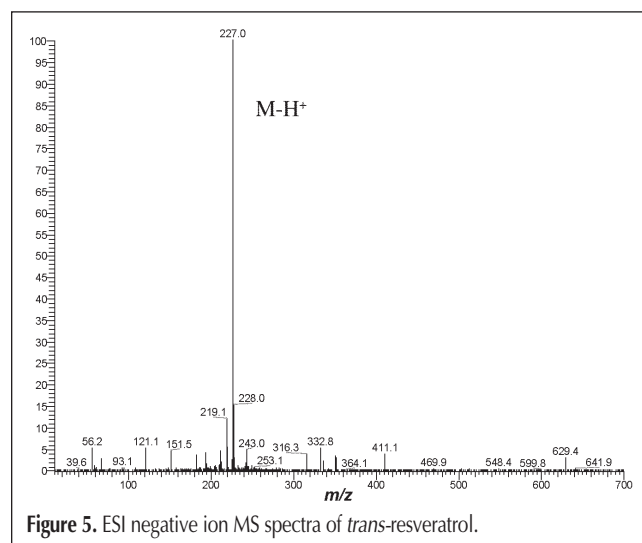


Figure 5. ESI negative ion MS spectra of *trans*-resveratrol.

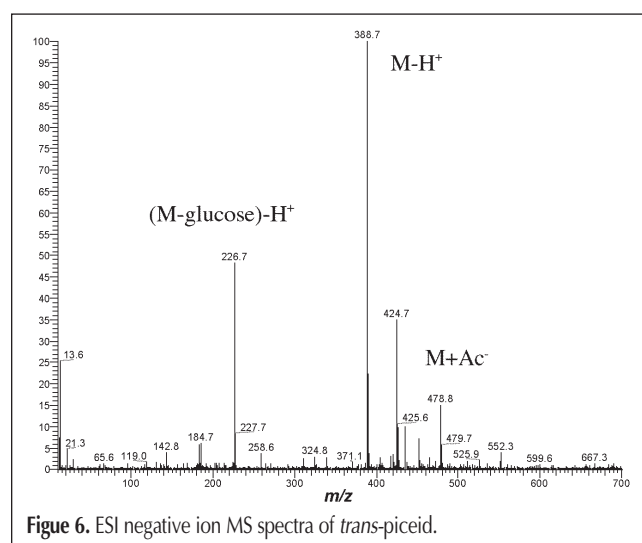


Figure 6. ESI negative ion MS spectra of *trans*-piceid.

and ESI ionization source were used, the probe temperature was 250°C, and the corona voltage or probe voltage was 3.5 kV. Spectra were recorded by 1.2 scan/s in the negative ion mode between m/z 10 to 700. The scan filter on the quadrupole analyzer was 10 and 20 V. Finnigan Xcalibur (version XCALI-97006) (San Jose, CA) was used to acquire the mass spectra of the compounds (Figures 3–6).

Results and Discussion

The separation and determination of *trans*-resveratrol and *trans*-piceid from wine with the proposed method is simple, sensitive, reproducible, and needs no sample pretreatment.

Validation of the method

Limit of detection

The limit of detection (LOD) for the new procedure (defined as the amount of analyte giving a peak height three times of the noise level) was very low. Using UV detection, the LOD was 0.9 pmol (205 pg) for *trans*-resveratrol and 0.6 pmol (234 pg) for piceid. Using MS detection, the LOD was even lower, at 0.3 pmol (68 pg) and 0.2 pmol (78 pg), respectively ($n = 5$).

Linearity

Over the selected concentration range, a linear relationship

Sample	Linear range (nmol \times cm ⁻³)	Correlation coefficient	Slope (x, y)*	Intercept
<i>Trans</i> -resveratrol	0.044–440	1.000	1.1415	-0.0117
<i>Trans</i> -piceid	0.025–250	0.9994	0.5622	+0.1414

* x = amount of sample (pmol) and y = peak area (mAU \times min).

Sample	Retention time (min)	SD*	Area (mAU \times min)	SD
Wine	21.853	0.039	4.6190	0.0212
Standard	21.684	0.024	4.5996	0.0274

* Standard deviation.

Sample	Retention time (min)	SD	Area (mAU \times min)	SD
Wine	18.013	0.087	6.3713	0.1227
Standard	18.200	0.172	0.4505	0.0066

was obtained between the peak area and concentration (Table I). Calibration was carried out at nine different concentrations of *trans*-resveratrol and *trans*-piceid standard solutions in the range of 0.01–101 mg/L. All injections were repeated three times ($n = 3$). The calibration ranges selected adequately covered the variations in the amounts of resveratrol and piceid in the samples. The correlation coefficients (R^2) were 1 and 0.999; the regression analysis demonstrates an excellent relationship between the peak area and concentration.

Repeatability and intermediate precision

Repeatabilities (intra- and interday precision) of the method were evaluated by assaying five replicate injections of a standard solution and a wine sample. The mean values and standard deviations of retention time and relative peak area are listed in Tables II and III. The standard deviations proved the accuracy and reproducibility to be very good.

Recovery

The recovery of the method was determined by the standard addition method on selected wine samples (Polgár Cabernet Sauvignon 2002 and Polgár Chardonnay 2003). In spiked samples ($n = 3$) the concentrations of the *trans*-resveratrol and *trans*-piceid were increased by 50%, 100%, and 150%. These spiked wine samples were analyzed ($n = 5$), and the amount of analyte recovered was calculated. The recovery data presented in Tables IV and V are satisfactory. The recoveries for the two important polyphenols were between 93.8% and 100.8% (mainly between 95% and 97%).

Sample	Amount of analyte (mg/L)		Recovery (%)
	Added	Measured	
Red wine	5.24	4.96	94.7
	10.48	10.02	95.6
	17.00	17.14	100.8
White wine	4.07	3.92	96.3
	8.15	7.79	95.6
	16.30	16.24	99.6

Sample	Amount of analyte (mg/L)		Recovery (%)
	Added	Measured	
Red wine	5.00	4.84	96.8
	10.23	9.83	96.1
	16.57	16.30	98.4
White wine	5.00	4.69	93.8
	10.65	10.17	95.5
	16.78	15.97	95.2

Table VI. The Trans-Resveratrol and Trans-Piceid Concentrations (mg/L) in Wine Samples

No.	Winery	Variety	Vintage year	Trans-resveratrol (mg/L)	SD (%)	Trans-piceid (mg/L)	SD (%)
1	Polgár	Portugieser	2000	0.43	3.8	4.45	0.7
2	Polgár	Kadarka	2000	0.10	4.9	4.37	4.6
3	Polgár	Zweigelt	2001	2.47	0.9	5.11	1.6
4	Polgár	Merlot	2001	3.04	1.3	4.84	0.7
5	Polgár	Cabernet Sauvignon	2001	2.72	0.7	8.34	0.6
6	Polgár	Cuvée	2001	1.36	4.9	6.76	4.1
7	Polgár	Cab Sauv/Cab franc	2001	1.35	4.4	7.16	3.6
8	Polgár	Portugieser	2002	1.94	0.9	9.46	0.2
9	Polgár	Pinot noir 2	2002	3.57	1.1	9.36	2.3
10	Polgár	Pinot noir 1	2002	3.73	1.1	9.09	0.1
11	Polgár	Zweigelt	2002	4.65	0.3	7.60	0.3
12	Polgár	Rubin Cuvée	2002	6.12	1.4	8.00	5.0
13	Polgár	Merlot	2002	14.32	2.3	6.01	1.6
14	Polgár	Cabernet Sauvignon	2002	9.34	0.7	7.70	0.8
15	Polgár	Rubin Cuvée	2003	2.34	3.2	9.32	0.1
16	Polgár	Oporto	2003	0.33	1.9	9.54	0.1
17	Polgár	Zweigelt	2003	0.56	4.2	7.63	1.0
18	Polgár	Kadarka	2003	1.68	1.3	6.43	0.1
19	Polgár	Kékfrankos	2003	2.23	0.7	7.73	0.5
20	Polgár	Merlot	2003	2.28	4.2	8.45	0.4
21	Polgár	Cabernet Sauvignon	2003	1.18	1.4	6.60	4.7
22	Polgár	Chardonnay barrique	2002	0.78	1.9	6.32	0.9
23	Polgár	Chardonnay barrique	2003	0.20	1.7	2.96	0.2
24	Polgár	Olaszrizling	2002	0.71	2.8	6.28	0.4
25	Polgár	Shyraz	2003	0.89	4.6	7.33	0.1
26	Polgár	Cuvée Barrique	2000	1.81	3.6	4.69	0.9
27	Polgár	Portugieser	2001	1.94	1.7	4.65	0.2
28	Polgár	Pinot noir	2001	2.84	3.4	8.16	0.1
29	Polgár	Cabernet Sauvignon	2001	2.72	0.7	8.54	0.1
30	Polgár	Csal válog Cab Sauv	2000	2.70	0.4	5.89	0.2
31	Polgár	Merlot	2000	2.03	0.6	7.95	0.3
32	Polgár	Zweigelt	2000	3.06	0.8	5.59	0.1
33	Polgár	Rubin Cuvée	2000	0.75	4.3	5.28	0.2
34	Polgár	Kékfrankos	2002	5.57	2.2	5.93	0.4
35	Bock	Kékfrankos	2002	2.79	1.1	6.04	0.3
36	Bock	Cabernet Sauvignon	1999	1.58	1.5	7.35	1.0
37	Bock	Merlot barrique Spec.	1997	2.11	1.7	6.00	0.1
38	Bock	Royal Cuvée	2000	1.07	3.7	4.50	0.8
39	Bock	Royal Cuvée	1999	2.16	1.4	5.10	0.2
40	Bock	Royal Cuvée	1996	2.05	0.3	4.69	0.5
41	Bock	Cabernet franc	2000	0.88	1.8	6.32	0.7
42	Bock	Ermitage	2002	2.44	2.6	7.63	3.6
43	Bock	Royal Cuvée	1997	2.35	1.7	4.79	3.9
44	Bock	Cab Sauv/Cab Franc	2000	0.57	6.3	4.23	0.9
45	Bock	Cab Sauv/Cab Franc	1996	1.47	1.5	6.05	0.2
46	Bock	Cab Sauv/Cab Franc	1998	2.28	2.6	7.34	0.1
47	Bock	Cab Sauv/Cab Franc	1999	1.67	0.8	6.87	4.2
48	Bock	Merlot Spec Res	1999	1.81	2.6	5.04	4.3
49	Bock	Cabernet Sauvignon	2000	1.53	4.3	6.11	0.1
50	Bock	Merlot	2001	3.23	1.5	6.63	0.4
51	Bock	Cabernet Sauvignon	2002	2.75	2.8	7.70	1.2
52	Bock	Shyraz	2003	1.22	1.8	11.29	2.8
53	Bock	Merlot	2002	7.03	0.0	9.39	4.7
54	Bock	Cabernet Franc	2003	0.66	4.6	10.58	0.1

Conclusion

A new, isocratic reversed-phase HPLC method for assay of *trans*-resveratrol and piceid in wine has been developed and validated. The method needs no sample preparation for the separation. The results showed that the technique is very sensitive (LOD between 0.2 and 0.9 pmol), reproducible, and accurate. The sensitivity can be further increased using MS detection. The recoveries for the two important polyphenols are in the range of 93.8–100.8% and are reproducible. More than 70 samples from the Villány wine region (south Hungary) were examined for their *trans*-resveratrol and *trans*-piceid content (Table VI).

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Table VI. The Trans-Resveratrol and Trans-Piceid Concentrations (mg/L) in Wine Samples (Continued)

No.	Winery	Variety	Vintage year	Trans-resveratrol (mg/L)	SD (%)	Trans-piceid (mg/L)	SD (%)
55	Bock	Cabernet Franc	2001	1.13	0.7	14.11	0.2
56	Bock	Merlot	2003	2.21	1.7	6.25	2.0
57	Bock	Cabernet Sauvignon	2001	2.11	2.6	10.90	0.6
58	Bock	Kékfrankos	2000	1.10	2.2	4.18	0.3
59	Bock	Cabernet Franc	2002	1.19	4.6	6.76	0.1
60	Bock	Cabernet Sauvignon	2003	1.75	3.8	n.d.	–
61	Bock	Merlot	2000	1.28	0.9	3.77	0.1
62	Bock	Oportó	2003	1.18	4.6	7.89	0.6
63	Bock	Cabernet Franc	1999	1.00	2.3	6.02	0.8
64	Bock	Cuvée	2001	2.17	0.6	7.25	4.3
65	Bock	Cuvée Barrique	1997	1.53	1.5	4.99	5.4
66	Bock	Kékfrankos	2001	3.77	0.1	5.97	0.2
68	Bock	Oportó	2003	1.23	2.6	8.05	0.1
69	Bock	Kékfrankos	2003	1.38	0.8	8.60	0.1
70	Bock	Oportó	2002	1.99	2.6	16.40	0.2
72	Bock	Cuvée	2001	2.32	0.2	8.81	0.1
73	Bock	Pinot noir	2001	2.83	3.5	9.26	0.1

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