Evaluation of Hungarian Wines for Resveratrol by Overpressured Layer Chromatography

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Keywords

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Abstract

A method, including solid phase extraction sample preparation, overpressured layer chromatographic separation and subsequent densitometric evaluation, was developed for measurement of total resveratrol (*cis-* and *trans*-isomers) content of wine. The amount of resveratrol was determined in wine samples from different winemaking regions of Hungary. The total resveratrol was high in Hungarian red wines (3.6–11 mg/L), and much lower in white ones (0.04–1.5 mg/L).

1 Introduction

The stilbene derivative, *trans*-resveratrol (TR, *trans*-3,5,4'-trihydroxystilbene, **Figure 1**) is a phytoalexin accumulated in a large number of plant species to protect them from microbial infections (e.g. toxin producing fungi) [1] and abiotic stresses (e.g. heavy metal ions, UV light exposure) [2, 3]. TR is commonly present in grater portions in grapes and consequently in wines [4, 5]. *Cis*-resveratrol (CR), the geometric isomer of TR, does not occur in blue grapes, but it is formed during the storage of the wine, so it is present in wines usually in much lower concentration than TR [6, 7]. However, in some Brazilian wines the concentration of CR was five times higher than that of the *trans* isomer [8]. Earlier studies show that there is a significant difference in the TR content of white and red wines, due to the different wine-making technologies: red wines usually contain higher amount of TR than white ones [9, 10]. It was reported that, in contrast to the wines, there is no considerable difference between the respective parts of white and blue grape varieties in TR content [11].

It has been established that TR has diverse beneficial activities which can be divided into two main groups [12]. The first group includes chemopreventive (e.g. antimutagenic, antioxidant and cardioprotective) effects [13, 14], and the second one means the killing/inhibiting effects (e.g. antibacterial, antifungal and anticancer activities) [15, 16].

The production of TR in grape berries was first reported by JEANDET *et al.* in 1991 [4]. Since this time, several methods have been developed for determining resveratrol (R, *cis-* and *trans*) and other poliphenols (e.g. quercetin, catechin, epicatechin) in grape berries, juices and wines. Electroanalytical methods for determination of polyphenols are relatively sparse in the literature. Voltammetric techniques are used for evaluation of total and individual poliphenols. Pekec *et al.* [17] described a sensitive electroanalytic method for determination of TR in grape samples by using a cyclodextrin modified carbon paste electrode applying

differential pulse voltammetry. Airado-Rodriguez *et al.* [18] published a method for evaluation of TR with low detection limit using adsorptive stripping square-wave voltammetry at glassy carbon electrode.

The widely used methods are based on high-performance liquid chromatographic (HPLC) separation with UV [6, 19–22], photodiode array [23–26], fluorometric [27, 28], electrochemical [11, 29], or mass spectrometric [10, 30, 31] detection. Gas chromatography coupled with mass spectrometry (GC-MS) [32, 33,] and capillary electrophoresis (CE) [34–36] are less frequently used techniques for analysis of the polyphenolic components of wines and grapes.

In accordance with the scientific-technical progress, some of authors published recent years ultra performance liquid chromatographic (UPLC) methods for analysis of R and other polyphenolic compounds [37, 38].

Planar layer liquid chromatography provides the facility of the parallel analysis of samples, which can make faster the analysis, and requires less mobile phase than the HPLC, therefore, it is cheaper and more environment friendly. However, the thin-layer chromatography (TLC) is not widely used for analyses of R and other polyphenolic compounds of grapes and wines because of their limitations connected to the quality of separation and sensibility. Chen *et al.* [39] used TLC and fluorescence detection to determine the CR, and TR and piceid.

We developed measurement of R by overpressured layer chromatographic (OPLC) separation after liquid-liquid extraction sample preparation [40]. OPLC [41, 42] integrates the benefits of TLC and HPLC. It is a forced flow technique, using external pressure on TLC plate sealed on the edges and a pump system for the admission of mobile phase into the stationary phase. We compared TLC to an automatic OPLC technique for separation of stilbene isomers, implicating the R isomers, from wine [43]. The results clearly show that

OPLC is more efficient than TLC, giving better resolution and higher theoretical plate number.

In connection with the sample preparation of wines, usually liquid-liquid extraction (LLE) [44, 45] or solid phase extraction (SPE) [46–48] are applied. SPE is very useful with its advantages such as high selectivity, reproducibility, facility of the preparation of more samples simultaneously, the automation of the process and it demands less solvent than LLE. Some authors published methods where samples are injected directly in the chromatograph without preparation [17–19, 49].

The aim of our study was to develop a relatively cheap and fast method for determination of total R in wines, using SPE sample preparation, OPLC separation and densitometric evaluation of chromatograms. We applied this method for analyses of some red and white wine samples from different wine regions of Hungary.

2 Experimental

2.1 Materials

TR was purchased from Sigma Aldrich Ltd. (Budapest, Hungary). Analytical grade solvents (Merck KGaA, Darmstadt, Germany) were used for solid phase extraction (SPE) and the water was deionized by Milli-Q$_{Plus}$, Ultra-Pure Water System from Millipore Co. (Billerica, MA, USA). Solvents used for OPLC were purchased from Reanal Ltd. (Budapest, Hungary). LiChrolut® RP-18 columns (40-63 μm; Merck KGaA, Darmstadt, Germany) with 500 mg sorbent was applied for SPE. Drying of SPE cartridges and solvent evaporation after SPE were carried out in a nitrogen stream (99.95%). Analytical OPLC was performed on 200 mm × 200 mm HPTLC silica gel 60 F$_{254}$ adsorbent plates (Merck KGaA, Darmstadt, Germany) sealed on all four edges [41].

2.2 Instrumentation

For SPE, a Lichrolut® extraction unit (Merck KGaA, Darmstadt, Germany) was employed, coupled to a water-jet vacuum pump. A Rotavapor R-134 (Büchi Labortechnik AG, Flawil, Switzerland) apparatus was used for evaporation of solvents under reduced pressure. OPLC separation [41, 42] for quantitative determination of R was performed by using an automatic OPLC 50 BS instrument (OPLC-NIT Ltd., Budapest, Hungary). Solutions were applied to the chromatoplates with a Linomat IV-Y sample applicator (Camag Co., Muttenz, Switzerland). Chromatograms were evaluated instrumentally with a Shimadzu CS-930 dual-wavelength TLC scanner (Shimadzu Co., Kyoto, Japan) at λ=310 nm.

2.3 Methods

2.3.1 Sample preparation

RP-18 SPE columns were used for sample preparation. The column had previously been activated with 5 mL methanol followed by 5 mL water. After the preconditioning, 5 mL of wine was applied to the column. The column was washed with 10 mL water at pH = 7.4 (adjusted with a buffer of K_2HPO_4 and KH_2PO_4), and dried with a nitrogen gas stream for 30 min. Elution was performed with 10 mL ethyl acetate. The eluate was evaporated in vacuum at 35 °C, and redissolved in 0.5 mL methanol.

2.3.2 Chromatographic separation

Samples were applied 30 mm from the edge of the layer. The other OPLC conditions were as follows: the mobile phase was chloroform–methanol (10:1, v/v), external pressure 50 bar, the rapid mobile phase flush 350 μL, development volume 8000 μL, the flow rate of the mobile

phase 350 µLmin^{-1}, and the development time was 1381 s [41]. In this system, CR and TR are

not separated, they were measured together. The development distance was 157 mm.

3 Results and Discussion

A method was developed for measurement of R including SPE sample preparation, OPLC

separation and densitometric evaluation. This SPE and the earlier demonstrated LLE [43]

sample preparations were compared. Besides of the mentioned advantages of SPE it also

provides clearer extracts, which can be observed in densitograms **(Figure 2)**. Because of the

better resolution (there is no disturbing peak) the adsorbent layer is more loadable, so the limit

of detection is lower in the case of SPE sample preparation (0.03 mgL^{-1}) than at LLE (0.08

mgL^{-1}).

The recovery was determined using a Cabernet Sauvignon red wine which contains 1.43 mgL^{-1} R. The wine was spiked with a methanol solution of TR test substance so, that the R content

was 3 mgL^{-1} high. Comparing the chromatograms in **Figure 2 (b** and **c)**, the difference can be

seen in the peak of R indicates the increase of the amount of R because of the spiking. The

recovery was 95 % (SD = 2 %, n = 3), which was calculated from the data included in **Table

1**. Data were obtained using the calibration curve **(Figure 3)**, which linearity was proved in

the range of 0.05–0.5 µg.

Wine samples were analyzed, originating from different traditional wine regions of Hungary

and from various vintages. Similarly to earlier studies [9, 10], high amounts of R were

measured in Hungarian red wines. The concentrations in R of red wines ranged from 3 to 13

mgL^{-1}. While in white wine samples, R showed lower concentration, typically from 0.1 to

0.25 mgL^{-1} **(Table 2)**. It was established that the concentration of R in wine depends on the

vintage, the varieties of grapes, the time of harvest, the making process [44, 50]. These

influences can cause the variances of the amount of R in the wine samples tested as well. However, the main reason for the difference between the amount of R in red and white wines can be originated from the winemaking technology, especially the fermentation on the skin, which is typical only for red wine.

TR is one of the active ingredients in red wines, which might contribute to the French paradox [51], that is the low incidence of coronary heart disease despite of high fat intake. It is known that the Hungarian cuisine is a bit fatty, and there are many people suffering in coronary heart disease in the country [52, 53]. It is possible to get well this status, because in the last years the amount of the red wine consumer was increased in Hungary and the experiments show high amount of R in Hungarian red wines. It has to be pointed out that besides TR, there are other stilbenes [54] and other biologically active components (e.g. flavonoids or terpenoids) [55, 56] in red wines responsible for different beneficial effects.

It can be said that the exploitation of the special advantages of OPLC technique can be carried out with the use an efficient sample preparation (e.g. SPE) and a modern quantitative evaluation. However, a logical effort is the further exploitation of this efficient planar layer liquid chromatographic technique for the separation of R and other ingredients from grapes and wines. It means e.g. the use of the flowing eluent wall (FEW) operating mode [57], the two-dimensional technical solution [58] and especially the biological detection possibility which can be solved only in the field of layer liquid chromatography [59].

4 Conclusion

Our results clearly illustrate the applicability of OPLC to the analysis of R in white and red wines on the basis of SPE followed by a simple separation on HPTLC silica gel layer. There

were significant differences between the R content of white and red wines, amounts varied between 0.04 (Chardonnay 2004, Eger) and 11.0 mgL^{-1} (Pinot noir P.1. 2002).

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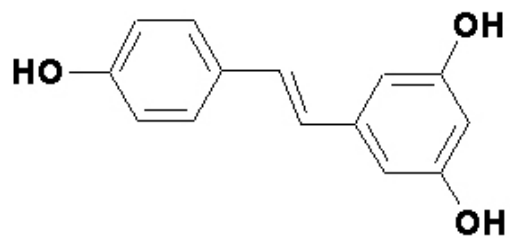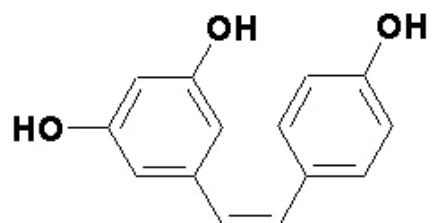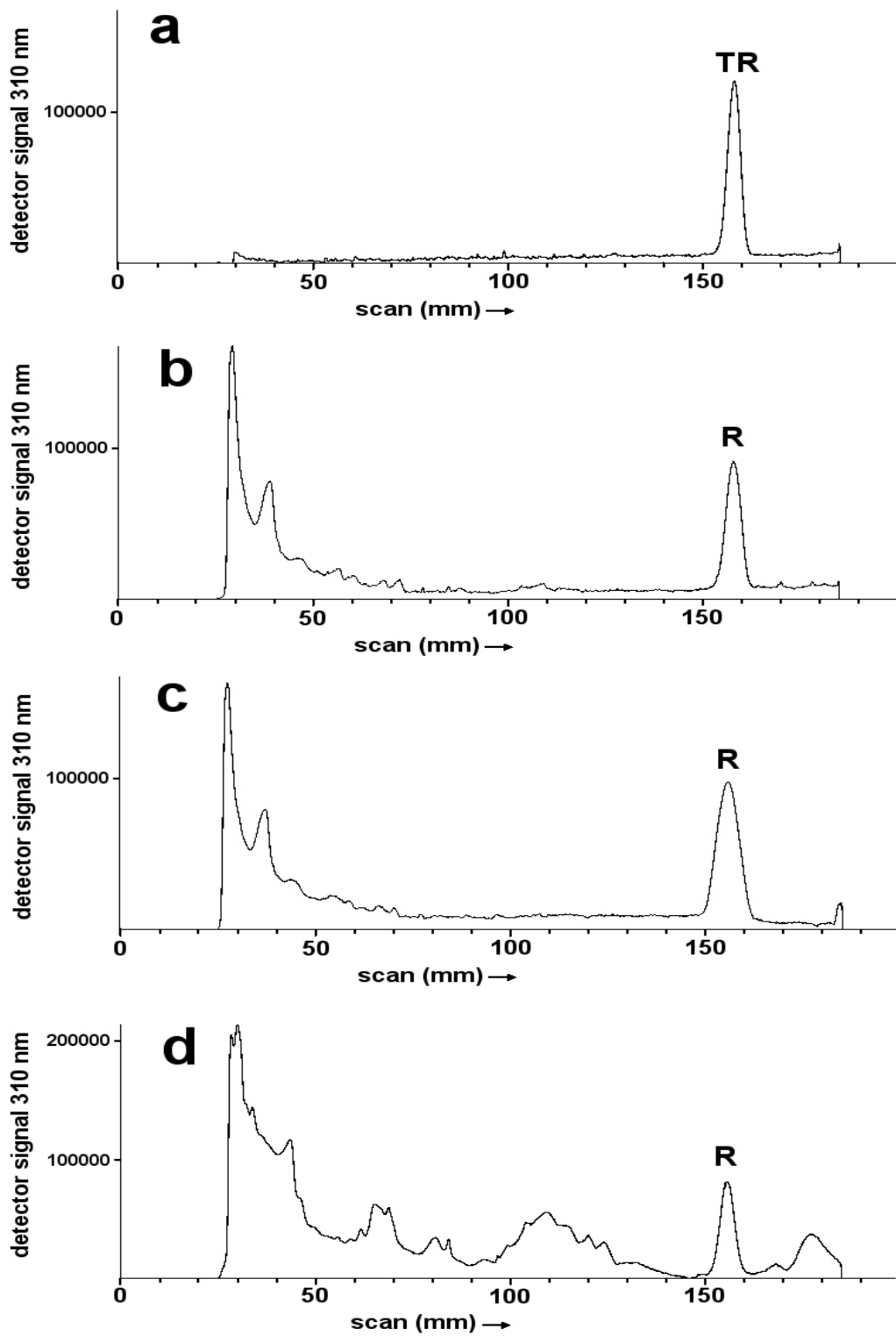
trans-resveratrol   **cis**-resveratrol

Figure 1

Figure 2

$$y = 4E+06x - 33928$$
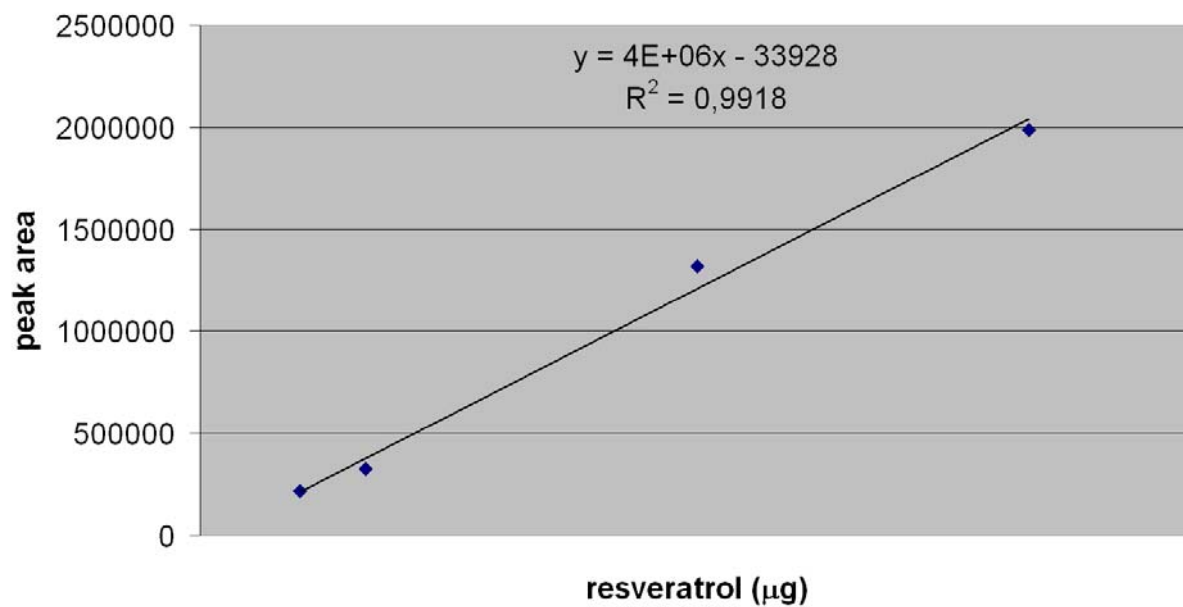$$R^2 = 0,9918$$

Figure 3

Captions to figures

Figure 1

Chemical structure of *trans*- and *cis*-resveratrol.

Figure 2

Densitograms obtained from: *trans*-resveratrol test substance, **a**; SPE sample prepared Cabernet Sauvignon red wine, **b**; SPE sample prepared and spiked Cabernet Sauvignon red wine, **c**; LLE sample prepared Cabernet Sauvignon red wine, **d**.

Figure 3 The calibration curve of resveratrol.

Table 1

Data for the recovery of R from wine in three parallels. 3 mgL^{-1} TR was added to a Cabernet Sauvignon red wine.

	R content of samples (mgL^{-1})			Average (mgL^{-1})	Standard deviation
	1	2	3		
I. Original cabernet Savignon	1.36	1.49	1.44	1.43	± 0.06
II. Spiked Cabernet Savignon	4.33	4.32	4.22	4.29	± 0.06
Differences (II.-1.43 mgL^{-1})	2.97	2.89	2.79	2.86	± 0.06
Recovery	97	96	93	95	± 2.00

Table 2

R content of some Hungarian red and white wines.

Wine samples	Amount of resveratrol (mgL^{-1})
Red wines	
Kékfrankos 2003, Villány	3.6
Blauburger 2002 Gyöngyösdomoszló	6.2
Cabernet Sauvignon 2002 Gyöngyösdomoszló	7.6
Pinot noir P.1. 2002 Gyöngyösdomoszló	11.0
Kékfrankos 2003 Eger-Tóbérc	8.5
Merlot 2003 Helvécia Great Plain	7.0
White wines	
Leányka 2001, Eger	1.5
Chardonnay 2004, Eger	0.04
Tramini 2003 Gyöngyösdomoszló	0.1
Hárslevelű 2003 Tokaj	0.2
Sauvignon Blance Gyöngyösdomoszló	0.09

The Location of figures and tables in the text

Location of **Figure 1**: first part of the chapter **Introduction**.

Location of **Figure 2**: at the beginning of the chapter **Results and Discussion**.

Location of **Figure 3**: the chapter **Results and Discussion**, after **Figure 2**.

Location of **Table 1**: the chapter **Results and Discussion**, after **Figure 3.**

Location of **Table 2**: the chapter **Results and Discussion**, after **Table1.**