

Full Research Paper

Investigation of Phenolic Components of Hungarian Wines

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Abstract: Ninety-two wines from the southernmost wine-producing region in Hungary (Villány) were analyzed for their polyphenolic content by high performance liquid chromatography (HPLC). Our results show that wine variety or vintage year could not be distinguished based on polyphenol content, but winery origin could be. Resveratrol concentration is mainly dependent on variety and vintage year. The “human factor” (i.e., winemaking style and technology) seems to be more decisive for the polyphenolic composition of red wines than other factors, such as variety and vintage year.

Keywords: wine, polyphenol, HPLC, phytoalexin

1. Introduction

Polyphenols are natural aromatic compounds containing two or more phenolic hydroxyl groups that occur in remarkable amounts in tissues seeds and fruits of several angiosperms [1-3]. They are

present in particular as defense agents facilitating protection against microbial infection and UV radiation. Several of their representatives have antioxidant effects, making their biological role even more important [4-7].

Phenolic compounds get into the human body as part of the diet. They decrease thrombocyte aggregation [8,9] and affect apoptosis and cell proliferation through modulation of signal transduction pathways [10]. Their antioxidant, anticarcinogen [11] and cardioprotective effects are also proven [12]. These properties are remarkable facts in light of the WHO mortality statistics: nowadays cardiovascular diseases are the leading cause of death in the developed countries, causing one third of the total mortality. Plant phenolics, however, are not only present in fruits and plants but they are also traceable in derived fruit products like white and red wines [13-15], determining their taste, colour and bitterness. These compounds in wines thought to account in large part for the so-called French-paradox [16-17]. This expression denominates the finding that the rate of coronary heart disease mortality in residents living in certain parts of France is lower than it would be expected, despite a diet prone to such events. This strong antioxidant, neuroprotective [18] and cardioprotective effect of polyphenols found in wines appears in even one thousand times diluted solutions as well. The importance of wine polyphenols lies in their good availability. In raw fruits phenolics occur as glycosides or as polymers, which results in equally limited digestibility and imbibitions. During the fermentation of red wine however these compounds shape into monomers. The approx. 10% alcoholic solution stabilizes the free ring compounds, so their imbibitions and digestibility increases.

The polyphenolic fingerprint might be a useful tool for the classification of wines. Several authors have shown that principal component analysis of the polyphenolic fraction can separate wines according to variety. A variety of techniques has been used to analyze the phenolic composition. Mainly HPLC is used for this purpose, sometimes connected to an MS, but other techniques, such as NIR and UV/Vis have also been successfully used [19-21].

Other authors have shown that polyphenolic fingerprints can not only be used to identify the geographic origin of wines, but even their winery origin and winemaking technology used. In one of our earlier studies on Hungarian red wines [22] we could not find evidence to support the last statement. Rather, our results showed a strong dependence of the polyphenolic composition on vintage year, but we could not distinguish among varieties based on the polyphenolic fingerprint.

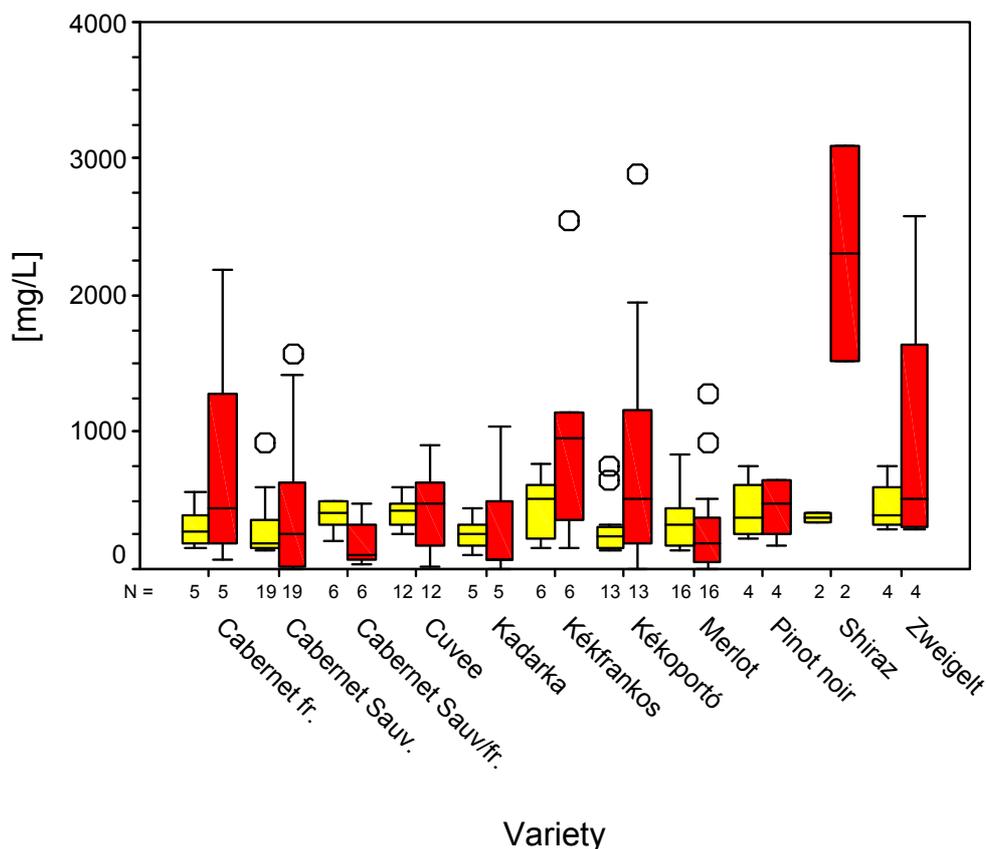
Furthermore, it is a well known fact, that premium wine production is limited to regions climatically conducive to growing grapes with balanced composition and varietal features [23]. Some preliminary results suggest that a positive correlation exists between location of the vineyard and stilbene concentrations of wines. A similar coherence can be observed between the high levels of stilbenes and quality-oriented grape producing practices [24,25].

2. Results and Discussion

Ninety-two wines from the vintages 1996-2006 from three different wineries from “Villány”, the southern-most wine-producing region in Hungary, were analyzed for their polyphenols contents by

HPLC using UV-VIS detection (Figure 1). Mean polyphenol content was highest in Kékfrankos (463 mg/L) and lowest in Kadarka (258 mg/L). Mean anthocyanin content was highest in Shiraz (2.309 mg/L) and lowest in Cabernet Sauvignon/Franc Cuvees (183 mg/L).

Figure 1. Polyphenol (yellow) and anthocyanin (red) content of red wines (n=92) from the Hungarian “Villány” region.



Detailed polyphenolic composition of the wines is given in table 1. The most striking fact is that Kékfrankos contains relatively high mean concentrations on colourless phenolics. Especially caftaric and caffeic acid, and (-)-epicatechin were found in high concentrations. A very interesting fact is that GRP could not be found in Kékfrankos. GRP is formed under oxidative conditions during winemaking from caftaric acid and glutathione [26]. Obviously, the generally high polyphenol content of this variety prevented the formation of GRP.

These values are, thus, in accordance with already published data. Kilmartin et al. (2002) found values between 3.1 and 107 mg/L for caftaric acid, 3.2 to 223 mg/L (+)-catechin, 2.4 to 143 mg/L (-)-epicatechin, 1.1 to 21 mg/L caffeic acid, and 1.2 to 13 mg/L quercetin in wines from New Zealand and Australia [27]. Rodríguez-Delgado et al. (2001) and Orbán et al. (2006) found similar concentrations in red wines from the Canary Islands and from the Hungarian wine region “Eger”, respectively [28,29]. Our data is also in accordance with the results of Californian and wines from the Spanish “Penedès” region as published by Ibern-Gómez et al. (2002) [30].

Using canonical discriminant analysis wines could be separated by origin (winery) based on their polyphenol content (Figure 2). These results show that different applied techniques during winemaking seem to have a much more decisive influence on the final polyphenolic composition of red wines than such things as variety and micro-climate, often also referred to as “terroir” [31-34].

Table 1. Polyphenolic composition [mg/L] of Hungarian wines; data represent means of two independent measurements.

Variety	N	Gallic acid		Tyrosol		Caftaric acid		(+)-Catechin	
		Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.
Cabernet franc	5	45.3	33.2	46.5	47.5	42.1	11.2	62.1	29.1
Cabernet Sauvignon	19	40.0	29.4	63.0	55.6	40.0	20.4	63.7	39.5
Cabernet Sauv/fr	6	70.9	22.6	54.9	33.9	54.8	13.0	69.0	29.9
Cuvée	12	62.5	16.4	78.4	35.6	55.2	14.6	74.7	23.9
Kadarka	5	32.1	24.7	33.5	43.0	45.5	19.1	82.5	42.9
Kékfrankos	6	46.0	10.1	82.9	63.2	87.0	26.7	71.5	33.0
Kékoportó	13	24.0	18.3	48.3	51.0	44.0	21.7	81.7	47.0
Merlot	16	51.0	27.3	62.7	47.2	38.9	23.0	72.1	41.0
Pinot noir	4	45.2	15.3	116.5	65.5	55.5	25.5	102.9	46.4
Shiraz	2	50.0	8.6	84.3	14.3	40.4	2.7	68.2	5.4
Zweigelt	4	58.3	11.4	86.7	77.7	77.5	7.5	73.4	50.5
Total	92	46.1	25.8	64.9	50.9	49.1	23.1	73.1	37.6

Variety	N	GRP		Caffeic acid		p-Coutaric acid		(-)-Epicatechin		p-Coumaric acid	
		Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.
Cabernet franc	5	3.5	4.9	14.6	14.3	0.0	0.0	92.0	76.5	9.2	6.7
Cabernet Sauvignon	19	0.8	2.6	13.9	11.7	4.5	4.3	63.6	62.8	4.6	5.8
Cabernet Sauv/fr	6	0.0	0.0	20.0	14.5	0.0	0.0	113.0	40.2	9.2	7.2
Cuvée	12	0.0	0.0	26.4	11.3	0.9	2.2	101.3	30.9	7.4	5.5
Kadarka	5	1.9	2.5	7.2	4.9	2.6	2.2	49.5	38.2	1.8	2.3
Kékfrankos	6	0.0	0.0	37.0	21.8	3.9	8.1	125.6	104.0	9.5	5.3
Kékoportó	13	1.7	3.8	20.5	17.4	4.8	4.9	55.0	59.6	3.5	4.4
Merlot	16	1.8	6.3	12.7	11.6	4.6	7.7	87.9	69.1	7.1	9.0
Pinot noir	4	0.0	0.0	28.9	16.4	0.7	1.4	64.6	68.5	8.9	8.7
Shiraz	2	0.0	0.0	24.4	2.3	4.8	6.9	99.7	35.6	8.3	3.8
Zweigelt	4	3.4	6.8	30.6	14.3	3.0	4.3	110.6	51.4	8.0	8.2
Total	92	1.2	3.7	19.4	15.1	3.2	5.1	82.4	62.8	6.4	6.6

Variety	N	Fertaric acid		Ferulic acid		trans-Resveratrol		Quercetin	
		Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.	Mean	Std.dev.
Cabernet franc	5	2.5	4.3	0.0	0.0	0.8	0.5	3.7	5.2
Cabernet Sauvignon	19	3.1	7.4	0.3	1.3	2.2	1.9	5.1	3.7
Cabernet Sauv/fr	6	0.9	2.3	0.0	0.0	1.2	0.8	2.4	3.1
Cuvée	12	3.3	6.7	0.3	1.2	2.2	1.3	6.1	4.9
Kadarka	5	1.3	3.0	0.8	0.8	1.0	0.8	5.7	4.0
Kékfrankos	6	0.0	0.0	0.0	0.0	2.8	1.7	11.3	5.0
Kékoportó	13	4.1	10.3	0.3	0.6	1.5	0.7	7.1	5.6
Merlot	16	2.4	6.3	3.0	5.9	3.4	3.2	8.4	10.0
Pinot noir	4	8.3	12.2	0.0	0.0	3.2	0.5	7.5	2.0
Shiraz	2	0.0	0.0	2.0	2.8	1.1	0.2	13.4	1.8
Zweigelt	4	7.9	9.3	1.9	2.3	2.7	1.7	6.0	6.3
Total	92	3.1	7.2	0.8	2.8	2.2	1.9	6.6	6.1

Variety	N	Delphi				Petuni					
		nidin-3-gluc		Cyanidin-3-gluc		din-3-gluc		Peonidin-3-gluc		Malvidin-3-gluc	
		Mean	Std.dev	Mean	Std.dev	Mean	Std.dev	Mean	Std.dev	Mean	Std.dev
Cabernet franc	5	63.4	72.8	0.0	0.0	73.0	96.5	39.3	44.4	656.2	696.2
Cabernet Sauvignon	19	53.5	62.1	2.2	6.4	41.9	46.2	24.8	27.1	314.0	381.4
Cabernet Sauv/fr	6	24.3	26.2	0.0	0.0	18.8	18.6	11.4	10.2	129.4	129.1
Cuvée	12	46.7	34.2	0.0	0.0	41.8	27.2	27.5	17.6	323.3	202.7
Kadarka	5	25.1	35.1	0.0	0.0	26.1	35.5	24.8	32.4	259.9	341.0
Kékfrankos	6	64.4	49.4	0.0	0.0	72.4	64.6	82.3	65.6	796.7	670.7
Kékoportó	13	39.1	42.0	0.0	0.0	53.8	56.2	34.9	38.6	638.0	752.2
Merlot	16	39.2	48.6	3.3	7.3	35.6	42.0	27.3	33.5	191.2	234.2
Pinot noir	4	43.0	22.8	0.0	0.0	39.9	24.6	48.1	35.6	316.1	156.2
Shiraz	2	151.8	43.4	0.0	0.0	220.2	109.3	127.9	42.7	1810.0	923.0
Zweigelt	4	71.5	74.6	0.0	0.0	88.0	84.8	61.7	61.8	753.9	867.2
Total	92	48.3	51.0	1.0	4.3	49.6	57.0	35.5	39.8	426.5	539.6

Trans-resveratrol content of Hungarian wines by grape varieties is strongly dependent on vintage and variety, which is consistent with our earlier studies [22]. As can be seen from figure 3, the 2002 vintage led to wines with high resveratrol content. According to winegrowing records in Hungary, 2002 has been an exceptionally good year with little to no fungus pressure in the vineyard. It is well known that high fungus pressure leads to destruction of resveratrol through the stilbene oxidase of the fungus *Botrytis cinerea* [25].

Figure 2. Canonical discriminant analysis of Hungarian red wines (n=92) from the “Villány” region based on their polyphenol content.

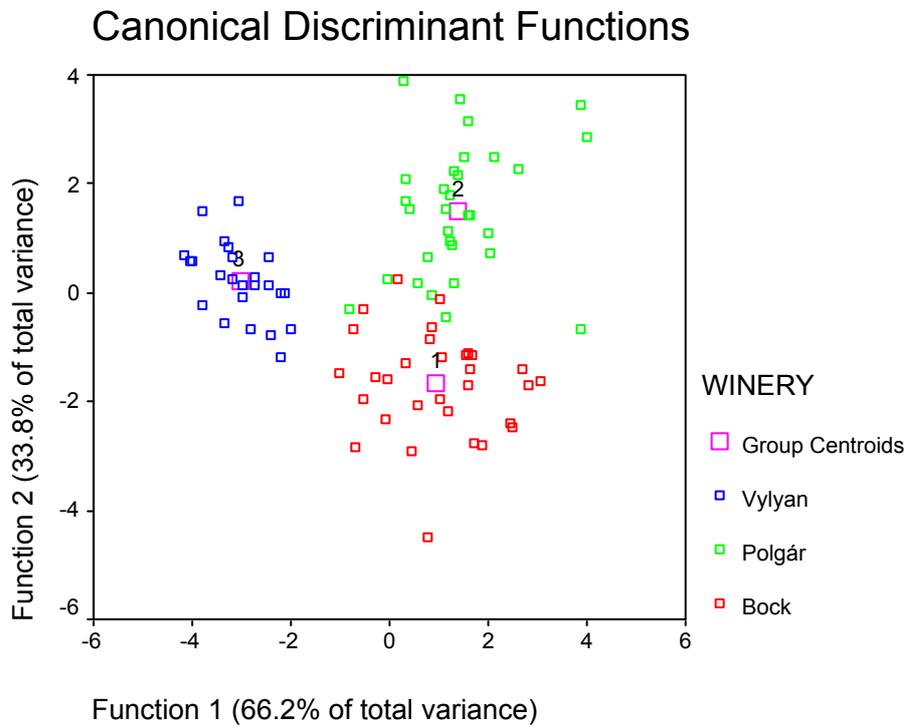
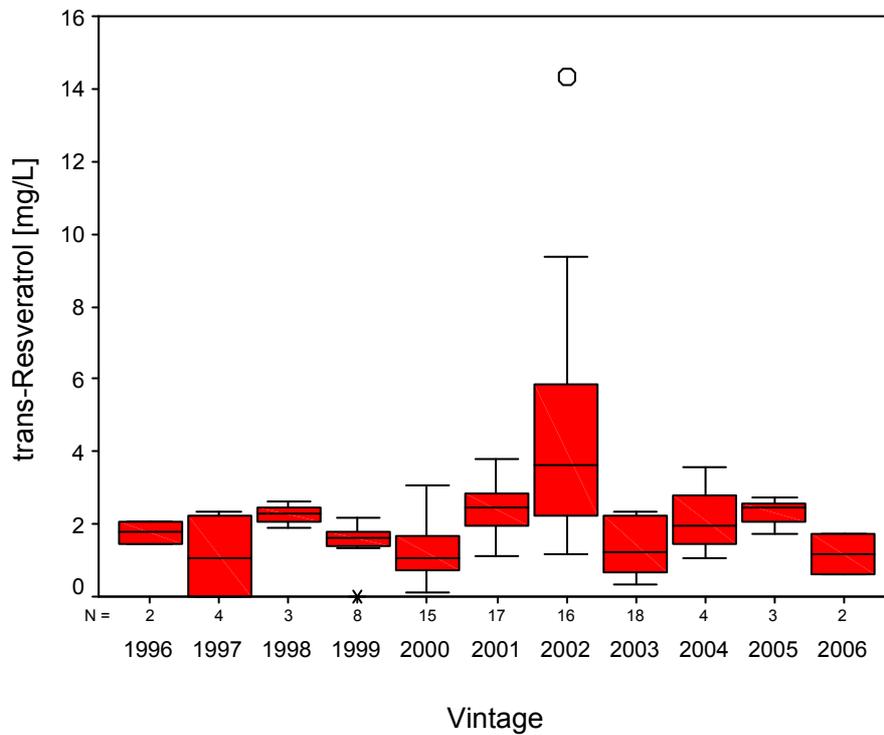
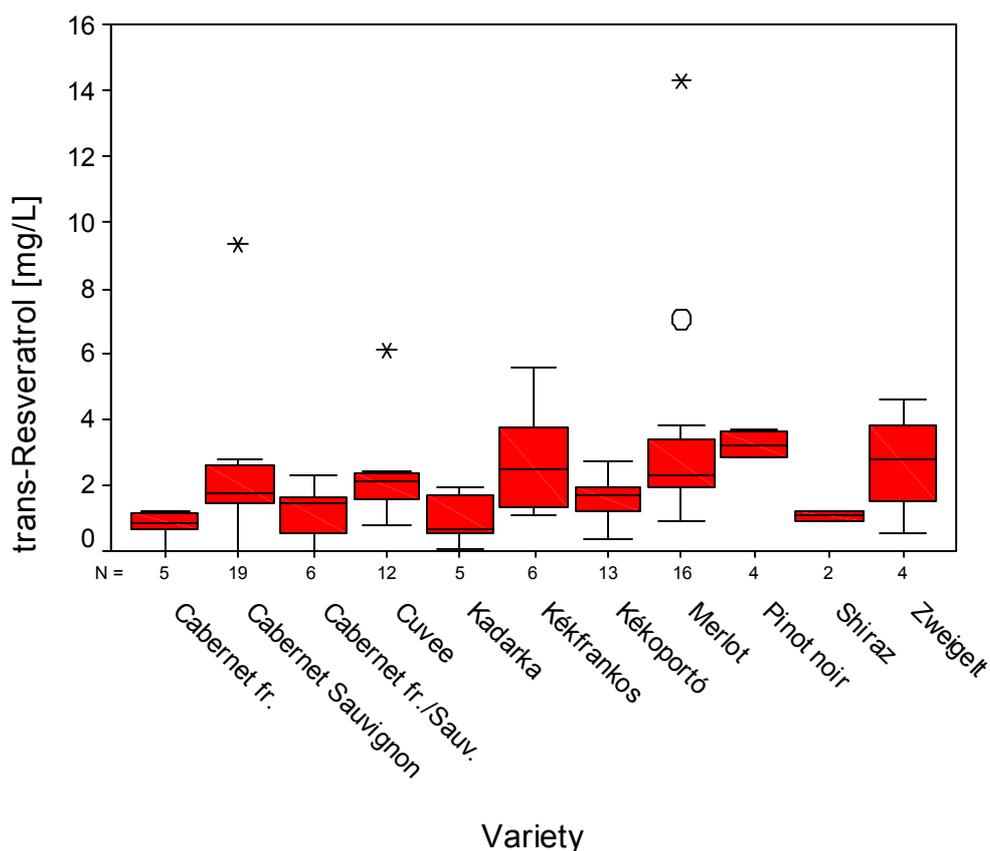


Figure 3. Trans-resveratrol content [mg/L] of Hungarian red wines (n=92) of the “Villány” region according to vintage year.



The highest concentrations on resveratrol were found in Merlot, Pinot noir, Kékfrankos, and Cabernet Sauvignon (ref. figure 4). Other studies have already shown that especially wines made from grapes of the Pinot family show especially high contents on resveratrol [22,32]. Our results are, thus, in accordance with earlier published data.

Figure 4. Trans-resveratrol content [mg/L] of Hungarian red wines (n=92) of the “Villány” region according to variety.



The concentration of *trans*-resveratrol seems also to be dependent on winery origin as already published by Bavaresco (2003). In our studies especially high concentrations were found in the wines from the Polgár winery.

Table 2. Trans-resveratrol [mg/L] in Hungarian red wines from the “Villány” region.

WINERY	Mean	N	Std. Deviation	Minimum	Maximum
Bock	1.87	37	1.1985	.00	7.03
Polgár	2.90	31	2.8447	.10	14.32
Vylyan	1.72	24	0.9414	.00	3.81
Total	2.18	92	1.9319	.00	14.32

According to statistics there is a highly significant dependency of *trans*-resveratrol content on winery origin ($p = 0.004$), which supports the results obtained from the canonical discriminant analysis that polyphenolic content is dependent on winery origin.

Materials and Methods

Chemicals & wine samples

All polyphenol standards were from Sigma-Aldrich Co. All reagents used were of analytical grade unless otherwise stated, acetic acid (gradient grade) and methanol from Brenntag. All of our samples were kept between 2 and 8 °C, but measured at a tempered workplace after a warming up period.

Polyphenol analysis

We applied two different HPLC methods, both were published previously [22,35]. Briefly, a Perkin–Elmer (Wellesley, USA) 200 Series HPLC system, consisting of degasser, autosampler, pump, column oven and PDA detector, was used. Injection volume was 20 µl. A ChromSep (LiChrospher) RP-18 end-capped 250 · 4.6 mm, 5 µm (Varian, Budapest, Hungary), column was used for separation and kept at 30°C. Chromatograms were recorded at 280 and 520 nm. The gradient consisted of two eluents: (A) water/phosphoric acid (99.5/0.5; v/v); (B) acetonitrile/water/phosphoric acid (50/49.5/0.5; v/v/v). Flow rate was 1.0 ml/min. Separation of components was achieved as follows: the concentration of A was kept constant for 2 min, then the concentration of B was increased over 5 min to 20%, then further increased to 40% over 18 min, followed by a hold of 6 min. B was increased to 80% over 4 min and then to 100% over 5 min, followed by a hold of 2 min. Equilibrium time to original conditions was 15 min. Some polyphenols were calculated as their respective free acids: caftaric acid, coutaric acid, fertaric acid, and GRP (grape reaction product, 2-*S*-glutathionyl caffeoyl tartaric acid). All anthocyanins were quantified as malvidin-3-*O*-glucoside.

Resveratrol analysis

The HPLC system for *trans*-resveratrol analysis consisted of a Gynkotec (Germering, Germany) M 480 GT pump, a Rheodyne 8125 (20 µl loop) injector and a Gynkotec M 340 S UV diode array detector. A 250 · 4.6 mm column, packed with 6 µm particle size C₁₈ material was used for the separations. A Chromeleon (Softron GmbH, Germering, Germany) data management software system was used for the control of the equipment and for data evaluation. Quantification was carried out using the peak areas method. A multi-step gradient method was applied, using methanol–water–acetic acid (10/90/1; v/v/v) mixture as solvent A and methanol–water–acetic acid (90/10/1; v/v/v) mixture as solvent B at a flow rate of 1.5 ml/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; 25.0–27.0 min, 100% B. Chromatographic separations were monitored at 306 nm. Detailed information on the validation of the method can be found in [35].

Statistical analysis

All statistical analysis was performed using Microsoft Excel® (Microsoft Corp., Redmond, USA) and SPSS® (SPSS Inc., Chicago, USA).

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