Developing and validation of an HPLC-DAD method for the determination of eight phenolic constituents in extract of different wine species

INTRODUCTION

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initation or propagation of oxidizing chain reactions[1]. Phenolic components being secondary metabolites are synthesized by different plants during regular development and show significant anioxidant activities and free radical scavenging properties[2-5]. Epidemiological studies showed that consumption of a healthy diet high in fruits and vegetables increased significantly the antioxidant capacity of plasma[6]. Furthermore, these studies showed that there is an inverse relationship between the intake of fruit, vegetables and cereals and the incidence of coronary heart diseases and certain cancers[7, 8]. The same relationshsip was proposed for wine consuming by different researchers[8-13]. Different fruits and vegetables show antioxidant properties[1, 2, 7, 14]. Among the natural antioxidants, red grape and its product wine have received much attention due to the high concentration and great variety of phenolic compunds[5, 8].

Winemaking is one of the most ancient of man's technologies, and known since the dawn of civilization and has followed human and agricultural progress on the world[15]. The earliest biomolecular archaeological evidence for plant additives in fermented beverages dates from the early Neolithic period in China and Anatolia. They had used different type of fruits and cereals to make their wine like grape, rice, millet and fruits[15, 16]. In earlier years in Egypt, a range of natural products specifically; herbs and tree resins were served with grape wine to prepare herbal medicinal wines[17]. Many of the polyphenols and other bioactive compounds in the source materials are bonded to insoluble plant compounds. The winemaking process releases many of these bioactive components into aqueous ethanolic solution, thus making them more biologically available for absorption during consumption[18]. Thus, winemaking is used to release benefical components such as phenolic compounds of the antioxidant fruits beside grape. There has been increasing interest on fruit wines produced different type of fruits. A non-grape fruit wine is a mixture composed of fruit juice, alcohol, and a wide range of components that may already be present in the fruit or synthesized during the fermentation process[19].

The antioxidant potential of wine is closely related to its phenolic content, which may be affected by a number of factors, including grape variety, fermentation processes, vinification techniques, ageing, and geographical and environmental factors (soil type and climate)[20]. According to the literature, there are different methods determining phenolic contents of the different wine samples such as high performance liquid chromatography – mass spectrometry (HPLC-MS)[3,8, 10, 21-23], high performance liquid chromatography

– diode array detector (HPLC-DAD)[5, 9, 11, 12, 24-27], gas chromatography (GC)[19], capillary electrophoresis (CE)[28], spectrophotometric[4,14,29,30], electrochemical methods[9, 31]. These methods come with some advantages and disadvantages. The important point at this situation, there is no paper about comparison of the phenolic profile of some local wine and fruit wines. In this study, a development and validation of HPLC-DAD method was presented to evaluate the phenolic profile of some selected Anatolian wines and fruit wines.

MATERIAL AND METHODS

Chemicals and Reagents

Standard materials of gallic acid (149-91-7) (**1**), chlorogenic acid (327-97-9) (**2**), epigallocatechin (989-51-5) (**3**), caffeic acid (331-39-5) (**4**), vanillin (121-33-5) (**5**), p-coumaric acid (501-98-4) (**6**), rutin (207671-50-9) (**7**) and quercetin (6151-25-3) (**8**) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Ortho-phosphoric acid (85%) solution, ethanol (HPLC gradient grade) and methanol (HPLC gradient grade) were from Merck (Darmstadt, Germany).

Ultrapure water for preparation of mobile phase (18.2 MΩ.cm at 25°C) was obtained by using Millipore Simplicity UV apparatus (Millipore , Molsheim, France).

Calibration, linearity, and quality control samples

The eight analytes stock solutions were prepared by dissolving weighed amount of the standard substance in ethanol at 1mg/mL concentration value. All stock solutions were stored in a refrigerator at 4°C. Combined working solutions of mixed analytes at the concentrations of 5, 10, 20, 50, 100 µg/ml were obtained by dilution of appropriate volume of stock solutions in volumetric flasks. Calibration curves were plotted, in triplicate, by analysing these standard solutions prepared freshly. Concentration values of the quality control samples (QC) were as follow: Low level concentration was 7.5 µg/ml, medium level concentration was 30 µg/ml and high level concentration was 80 µg/ml for each analyte.

Instruments and chromatographic conditions

Chromatographic analyses of phenolic compounds were performed by using Agilent 1260 HPLC system consisting of a quaternary pump model G1311B, an auto injector model G1329B, a thermostated column compartment model G1316A and a diode array detector (DAD) model G4212B. The chromatograms were monitored and integrated by using Agilent ChemStation software. Chromatographic separations of analytes were achieved on an Agilent Zorbax Eclipse XDB- C18 column (4.6 mm x 150 mm, 3.5 µm particle size) and the column was thermostated at 25±1 °C during analysis. DAD signals for every analyte were selected acoording to their spectrums obtained from Agilent ChemStation Software. Appropriate wavelenghts were selected as: 214 nm for gallic acid, chlorogenic acid and quercetin, 306 nm

for vanillin, p-coumaric acid and rutin, 333 nm for chlorogenic acid and caffeic acid. Gradient elution system was used to separate all analytes. For this purpose two different mobile phase were used; Mobile phase A was 10mM phosphoric acid solution and mobile phase B was methanol using a flow rate of 1ml/min. The optimised gradient programme was as follows: 0–15 min (0-60% B), 15–20 min (60–80% B), 20.0–22 min (80-100% B), 22–27 min (100–0% B) and 27–32 min (0% B). Samples were injected into the system as 10 µl.

Preparation of wine extracts

Both fruit wines and grape wine of Papazkarasi type cultivar were purchased from local producers in Turkey. After removal of alcohol by using a rotatory evaporator, the residual part of each wine was lyophilized by Christ Alpha 2-4 LD lyophilizator. The lyophilized extracts were dissolved in water at proper concentrations prior the experimentation.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

To achieve the best separation different mobile phases were investigated like buffers, organic solvents and different concentrations and different mixtures of these solutions. For the reason of all substances analyzed should be in non-polar form, the analysis media was preferred as acidic. For this purpose, acetate buffer, phosphate buffer solution and phosphoric acid solution was tried. The best separation performance was observed, when phosphoric acid solution was used. The concentration of the phosphoric acid was investigated as allowed as column filling material properties. Beside of concentration affect, organic modifier effect was investigated by using methanol and acetonitrile. During this process, peak shape, peak heigth and separation ability of the investigated system were evaluated. It was seen that 10 mM phosphoric acid solution was the most appropriate solution with methanol to separate eight different phenol compounds. After determining the mobile phase components, different mixture of these solutions at different rates were tried to achieve the best separation for all analytes by isocratic elution. But gradient elution provided both the best separation of all analytes and optimum analysis time. Therefore, 10 mM phosphoric acid solution was used as mobile phase A and methanol was used mobile phase B for further experiments.

On the other hand, other chromatographic conditions like flow rate, injection volume and temperature were investigated. At the end of experiments optimum parameters were determined as 1 ml/min for flow rate, 10 µL for injection volume and 25°C for temperature providing the best separation of eight phenolic compounds. Chromatogram showing separation of all analytes at optimized conditions is presented in Figure 1. As seen in this figure all analytes were separated from each other well and can be observed individually.

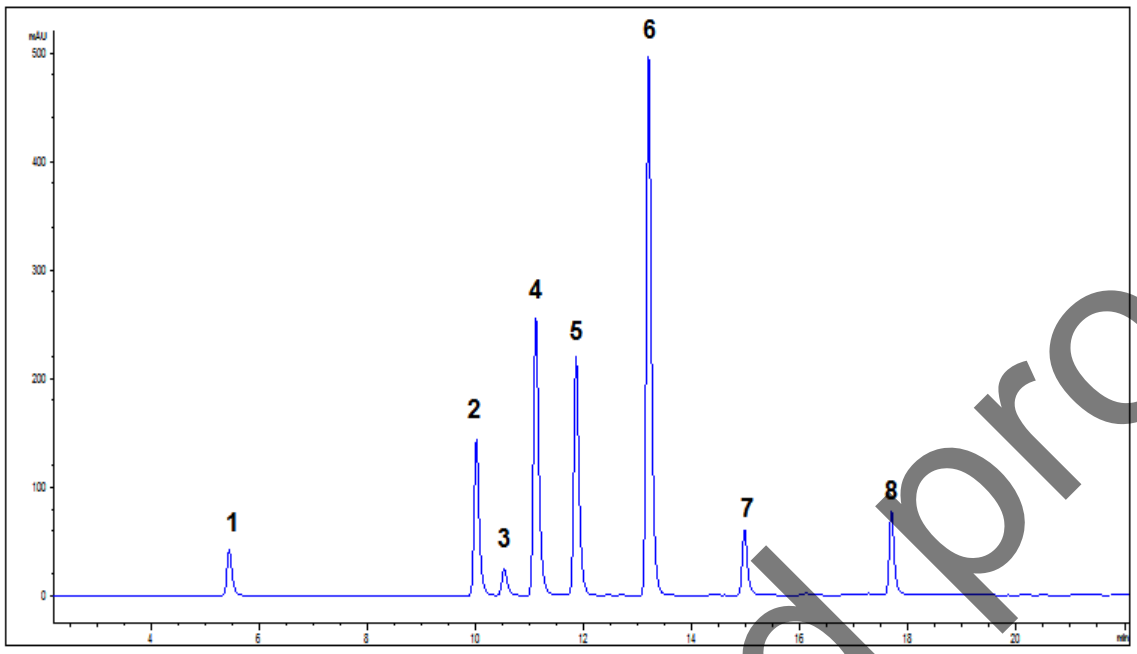

Figure 1. Obtained chromatogram of the 80 ppm standard mixture at 306 nm wavelength by using developed and optimized HPLC-DAD method. Gallic acid (**1**), chlorogenic acid (**2**), epigallocatechin (**3**), caffeic acid (**4**), vanillin (**5**), p-coumaric acid (**6**), rutin (**7**) and quercetin (**8**)

Method Validation

System Suitability Test

Before performing any validation experiments, researcher should establish that the HPLC system procedure is capable of providing data of acceptable quality[32] and make a test naming as system suitability test. System suitability is widely recognized as a critical component in chemical analysis and is frequently referred to in governmental regulations and guidance policies[33]. These tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. Parameters related with system suitability test are investigated as follow: plate count (N) should be higher than 2000, tailing factors (T) should be equal or lower than 2, resolution (R) between two peaks should be higher than 2, RSD value of retention time and area for six repetitions as repeatability should be equal or lower than 1% and capacity factor (k') should be higher than 2[32].

In the light of this information, system suitability test results were investigated before validation studies. For this purpose, a standard mixture was preprared which was containing 7.5 µg/mL of gallic acid, chlorogenic acid, epigallocatechin, caffeic acid, vanillin, p-coumaric acid, rutin and quercetin. Six replicate analysis of this standard mixture was performed. All results obtained from chromatograms are shown in Table 1. It can be seen that all results were in the appropriate range and optimized method was appropriate to apply validation process.

Table 1. System suitability test results for 7.5 µg/ml of standard mixture (n=6)

Parameter	1	2	3	4	5	6	7	8
Retention Time (min)	5.452	10.015	10.547	11.119	11.857	13.181	14.936	17.663
k'(≥2)	4.472	9.08	9.607	10.171	10.914	12.268	14.056	16.785
USP Tailing (≤2)	0.635	0.785	1.324	0.923	1.101	1.139	1.016	1.285
N, Theorethical Number (≥2000)	7669	40347	55460	42850	55837	72625	91738	148468
Resolution (≥2)	20.37	2.763	2.845	3.548	6.785	9.018	14.234	30.749
RSD (≤1%)	0.050	0.030	0.031	0.027	0.027	0.024	0.023	0.014

Calibration Curves

Different concentration values of each phenolic compounds were investigated to determine dynamic range for the method developed. For this purpose, standard solutions of each analyte as a mixture were prepared daily by diluting from stock solution of compounds. Chromatograms obtained for each standard mixture were recorded and investigated to determine calibration parameters of the method.

Table 2. Calibration curve parameters of the method developed for each analyte

	1	2	3	4	5	6	7	8
LOD (ppm)	0.99	0.62	0.14	0.09	0.04	0.05	0.42	0.04
LOQ (ppm)	3.32	2.06	0.48	0.30	0.13	0.16	1.40	0.12
Range (ppm)	5-100	2.5-100	1-100	1-100	1-100	1-100	2.5-100	1-100
Slope	60.959	25.018	63.616	51.415	36.368	75.341	8.3925	46.750
S_b	20.220	5.165	3.0640	1.525	0.488	1.186	1.174	0.587
R^2	0.9988	0.9988	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999

Also limit of detection (LOD) and limit of quantification (LOQ) values of each substance were calculated by using calibration curve equations. As known, LOD value is calculated by using standard deviation of y-intercepts of regression lines. Sum of three times of this standard deviation value on intercept of calibration curve and intercept value corresponds to LOD signal value. As same way, sum of ten times of this standard deviation value on intercept of calibration curve and intercept value corresponds to LOQ signal value. Thus LOD and LOQ values can be calculated by using this approach. In this study, limits of method developed were determined by this calculation way.

Calibration curve dynamic ranges and related method limits are shown in Table 2.

Accuracy

Accuracy studies for the method developed were performed by three repetitive analyzing samples of known concentration at three different level as low, medium and high level in dynamic range. For this purpose, standard mixtures of each compund at three different concentration values were prepared by

diluting stock solution and concentration values were as 7.5, 30 and 80 µg/mL. After analyzing these standard solutions, results obtained were investigated and concentration values calculated were compared with known concentration values as recovery. This comparison was made both for intra-day studies and inter-day studies. Results are presented in Table 3.

When the Table 3 is investigated, it is seen that recovery values are in 95-105 % range. This situation shows that the method developed is an accurate method.

Table 3. Results of accuracy and precision study for the developed method

Analyte	Concentration Level	Intra-day variation		Inter-day variation	
		Accuracy	RSD	Accuracy	RSD
Gallic Acid	L	112.99	1.26	105.28	8.70
	M	104.74	1.35	103.32	6.88
	H	99.02	1.05	100.57	2.98
Chlorogenic acid	L	98.54	0.51	96.96	10.06
	M	117.74	0.25	109.64	9.08
	H	98.57	0.21	107.95	8.62
Epigallocatechin	L	104.99	0.51	102.00	9.09
	M	105.76	0.34	104.06	1.75
	H	98.59	0.24	99.66	0.94
Caffeic Acid	L	102.07	0.42	103.03	1.19
	M	99.38	0.08	99.38	0.14
	H	100.63	0.09	100.66	0.34
Vanillin	L	104.98	0.35	105.19	0.27
	M	100.25	0.06	100.44	0.16
	H	100.38	0.09	100.36	0.11
p-Coumaric Acid	L	104.80	0.30	104.98	0.15
	M	100.22	0.05	100.40	0.16
	H	100.49	0.10	100.48	0.09
Rutin	L	105.16	0.97	104.19	3.54
	M	100.38	0.23	100.71	0.54
	H	100.46	0.07	100.38	0.17
Quercetin	L	103.84	0.32	103.29	1.75
	M	101.42	0.67	101.20	0.22
	H	100.64	0.09	100.66	0.08

L :Low level QC(7.5 µg/ml) M:Medium level QC (30 µg/ml) H: High level QC (80 µg/ml)

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (RSD) for a statistically significant number of samples. Table 3 also shows precision of the method due to presentation of RSD values obtained from three repetitive analysis of known amount of standards at three different level. For the most of the components, these RSD values for intra-day studies were lower than 1% value that the method very precise in intra-day studies except for gallic acid. When RSD values for inter-day studies were investigated, it was seen that RSD values for gallic acid, chlorogenic acid and epigallocatechin were out of the limits. This situation indicates that, especially these three substance should be analyzed by using daily calibration system. Unfortunately, the method developed can not be precise for inter-day studies and analysts should

work carefully and preparing of standard solutions especially at low concentration values need more attention.

Specifity

The specifity of the method was demonstrated by using spiked wine extract samples. For this purpose each standard solution was spiked to same wine extract and analyzed. It was observed that materials being in wine extract samples do not present overlapping peaks with eight phenolic compounds. Also peaks observed were investigated by comparing UV spectrums obtained from chromatograms of standard solution and chromatograms of extracted wine samples.

Robustness and Ruggeddness

The robustness and ruggedness of the method were investigated by changing of the some analytic parameters deliberately in the range of ±10%. Investigated parameters were injection volume, temperature and concentration of phosphoric acid. Injection volume and temperature were parameters related with instrument and temperature was related with preparation of the mobile phase. Thus, both instrumental and personal error sources were investigated. Recovery values were calculated again for the new conditions and the results obtained are shown in Table 4. In general, when the obtained recovery values were investigated, it can bee seen that recovery values were appropriate to 85-115 % percentage rule. Especially at low concentration level recovery values were affected from the changes. It means that if the analyte amount in the sample was at low level, analyst should be more carefull on analysis. The obtained recovery values were in the range between 88-105 % which show that this method is robust.

Table 4. Obtained recovery values during robustness- ruggedness studies. Results were expressed as the mean of triplicates ± standard deviation (S.D.)

		Injection Volume		**Temperature**		**Concentration of phosphoric acid**	
Analyte	**Conc. Level**	9 µL	11 µL	23°C	27°C	12 mM	8 mM
Gallic Acid	L	92.03±1.20	97.42±0.20	104.00±2.01	98.50±0.92	91.93±0.44	89.86±0.79
	M	98.94±1.25	99.20±0.58	98.43±0.07	100.53±0.16	99.72±1.15	97.41±0.07
	H	99.80±0.25	99.42±0.09	99.38±0.10	99.99±0.10	101.45±1.23	104.08±0.26
Chlorogenic	L	100.43±3.41	99.99±1.84	96.74±1.22	96.20±0.92	92.48±0.05	90.65±0.09
Acid	M	101.42±2.85	98.01±1.32	99.22±0.05	99.26±0.13	98.23±0.11	97.34±0.06
	H	98.14±1.45	99.54±0.13	99.44±0.05	99.68±0.11	101.59±0.87	103.82±0.28
Epigallocatechin	L	95.95±2.21	103.02±1.44	97.13±1.19	96.70±0.92	91.37±0.06	90.30±1.58
	M	99.60±0.36	97.35±0.22	99.02±0.02	99.31±0.09	97.20±0.08	98.42±0.02
	H	98.45±0.25	102.33±7.46	99.37±0.04	99.59±0.05	101.18±0.66	101.43±0.32
Caffeic Acid	L	94.10±0.88	98.25±0.51	96.73±1.02	95.65±0.94	90.81±0.02	90.80±0.02
	M	99.79±0.40	99.07±0.58	99.22±0.02	99.24±0.09	97.98±0.16	97.88±0.16
	H	100.45±0.40	99.01±0.53	99.50±0.07	99.80±0.13	101.87±0.87	101.87±0.87
Vanillin	L	92.97±1.32	97.66±0.19	95.79±1.08	95.54±0.72	90.25±0.03	88.09±0.05
	M	100.28±0.50	98.95±0.62	99.16±0.07	99.32±0.12	97.97±0.10	96.93±0.05
	H	100.09±0.28	99.54±0.41	99.52±0.06	9.91±0.07	101.98±0.92	104.34±0.22
p-coumaric acid	L	93.21±1.56	97.43±0.36	96.24±0.85	95.74±0.95	90.80±0.17	88.89±0.05

	M	100.15±0.10	99.76±1.22	99.23±0.08	99.39±0.10	98.09±0.12	97.20±0.03
	H	99.83±0.18	99.36±0.13	99.49±0.05	99.78±0.12	101.85±0.86	103.98±0.25
Rutin	L	90.56±1.74	94.06±0.79	94.47±1.00	97.22±2.34	92.43±0.09	100.52±0.06
	M	101.21±1.06	99.64±1.23	99.35±0.03	99.16±0.06	98.44±0.13	96.65±0.02
	H	101.32±1.28	99.39±0.20	99.54±0.08	99.85±0.13	101.84±0.84	105.81±0.29
Quercetin	L	89.25±1.01	92.53±1.37	96.36±0.86	95.30±0.77	90.52±0.33	89.02±0.26
	M	99.28±0.95	99.42±1.25	98.84±0.02	99.23±0.43	97.97±0.15	97.18±0.05
	H	101.47±1.82	99.62±0.82	99.52±0.06	99.77±0.14	101.84±0.83	104.01±0.27

Analysis of Phenolic Compounds in Wine Extract Samples

The method developed and optimized was applied for analysis of eight different phenolic compound in different wine extract samples. One of the obtained chromatograms was presented in Figure 2. Tablo 5 shows the results for this analysis.

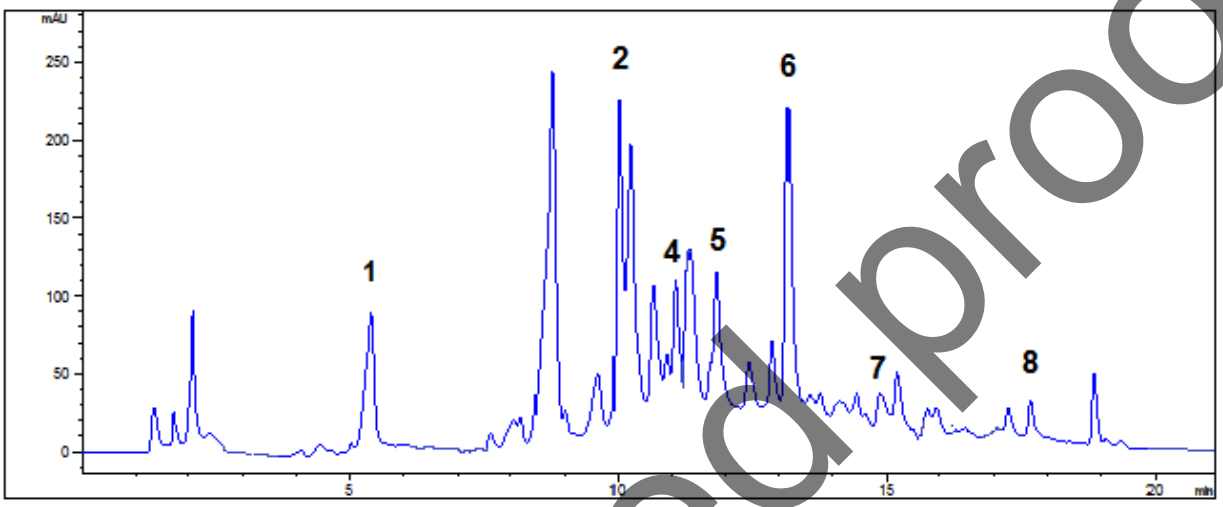

Figure 2. A sample HPLC chromatogramof black mulberry wine extract (visualized at 306 nm) Peaks: (**1**) Gallic acid; (**2**) Chlorogenic acid; (**4**) Caffeic acid; (**5**) Vanillin; (**6**) p-coumaric acid; (**7**) rutin; (**8**) quercetin.

When the analysis results were investigated, it was seen that epigallocatechin can not detect in these wine samples. If it is needed to make a comparison between the other phenolic compounds found in these wine samples, it can be understood that black mulberry contains phenolic compounds more than other wine samples. Celep et al. applied total phenolic content (TPC) and total antioxidant capacity (TOAC) tests to these wine samples and they showed that the black mulberry wine had the higher TPC and TOAC property than other wine samples. Analysis results of the wine samples support the these TPC and TOAC test results.

Table 5. Phenolic composition of the wine extracts by using the method developed. Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as µg/mg sample

Analyte	Blueberry Wine	Black Mulberry Wine	Cherry Wine	Papazkarası Wine
Gallic Acid	1.2 ± 0.070	1.66 ± 0.085	0.73 ± 0.014	0.20 ± 0.028
Chlorogenic Acid	n.d.	1.56 ± 0.096	n.d.	n.d.
Epigallocatechin	n.d.	n.d.	n.d.	n.d.
Caffeic Acid	0.06 ± 0.010	0.32 ± 0.003	0.12 ± 0.009	0.48 ± 0.080
Vanillin	0.02 ± 0.001	0.59 ± 0.016	0.02 ± 0.003	0.01 ± 0.003
p-Coumaric Acid	0.08 ± 0.017	0.55 ± 0.020	0.08 ± 0.002	0.09 ± 0.003
Rutin	0.33 ± 0.015	0.91 ± 0.012	0.19 ± 0.006	0.17 ± 0.005
Quercetin	0.08 ± 0.004	0.33 ± 0.008	0.08 ± 0.005	0.01 ± 0.001

CONCLUSION

This method developed and validated was applied succesfully to determine the phenolic constituents of the different wine samples. Obtained results were well-fitted with TPC and TOAC tests published previously. The method developed also used for the determination of the phenolic compounds of styrax liquids and different pekmez samples.

REFERENCES

1. Wei Zheng SYW. Antioxidant Activity and Phenolic Compounds in Selected Herbs. J Agr Food Chem. 2001; 49:5.
2. Kahkönen MP, Heikki Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J Agr Food Chem. 1999; 47:8.
3. Mirnaghi FS, Mousavi F, Rocha SM, Pawliszyn J. Automated determination of phenolic compounds in wine, berry, and grape samples using 96-blade solid phase microextraction system coupled with liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2013; 1276:12-9.
4. Stratil P, Klejdus B, Kuban V. Determination of Total Content of Phenolic Compounds and Their Antioxidant Activity in Vegetables- Evaluation of Spectrophotometric Methods. J Agr Food Chem. 2006; 54:9.
5. Milano F, Giannetti V, Gobbi L, Recchia L, Tarola AM. Simultaneous Determination of Phenolic Compounds in Selected Italian Red Wines. Journal of Commodity Science, Technology and Quality. 2009; 48(I):10.
6. Cao G, Booth SL, Sadowski JA, Prior RL. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr. 1998; 68:6.
7. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997; 2(4).
8. Lingua MS, Fabani MP, Wunderlin DA, Baroni MV. From grape to wine: Changes in phenolic composition and its influence on antioxidant activity. Food Chem. 2016; 208:228-38.
9. Šeruga M, Novak I, Jakobek L. Determination of polyphenols content and antioxidant activity of some red wines by differential pulse voltammetry, HPLC and spectrophotometric methods. Food Chem. 2011; 124(3):1208-16.
10. Dias FS, David JM, David JP. Determination of Phenolic Acids and Quercetin in Brazilian Red

Wines from Vale do São Francisco Region Using Liquid-Liquid Ultrasound-Assisted Extraction and HPLC-DAD-MS. J Brazil Chem Soc. 2016 ;.

11. Porgalı E, Büyüktuncel E. Determination of phenolic composition and antioxidant capacity of native red wines by high performance liquid chromatography and spectrophotometric methods. Food Res Int. 2012; 45(1):145-54.
12. Burin VM, Arcari SG, Costa LLF, Bordignon-Luiz MT. Determination of some phenolic compounds in red wine by RP-HPLC: Method Development and validation. J Chromatogr Sci. 2011; 49.
13. Peri P, Kamiloglu S, Capanoglu E, Ozcelik B. Investigating the Effect of Aging on the Phenolic Content, Antioxidant Activity and Anthocyanins in Turkish Wines. J Food Process Pres. 2015; 39(6):1845-53.
14. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 2007; 101(1):140-7.
15. Jagtap UB, Bapat VA. Wines from fruits other than grapes: Current status and future prospectus. Food Bioscience. 2015; 9:80-96.
16. Özdemir D. The demand for Turkish wine: estimates of the wine price elasticities. Appl Econ Lett. 2015; 22(17):5.

    McGovern PE, Mirzoian A, Hall GR. Ancient Egyptian herbal wines. P Natl Acad Sci USA. 106(18):7361-6 (2009).
17. Shahidi F. Nutraceuticals and functional foods: Whole versus processed foods. Trends Food Sci Tech. 2009; 20(9):376-87.
18. Amidžić KD, Klarić I, Mornar A, Nigović B. Evaluation of volatile compound and food additive contents in blackberry wine. Food Control. 2015; 50:714-21.
19. Celep E, Charehsaz M, Akyüz S, Acar ET, Yesilada E. Effect of in vitro gastrointestinal digestion on the bioavailability of phenolic components and the antioxidant potentials of some Turkish fruit wines. Food Res Int. 2015; 78:209-15.
20. Restivo A, Degano I, Ribechini E, Colombini MP. Development and Optimisation of an HPLC-DAD-ESI-Q-ToF Method for the Determination of Phenolic Acids and Derivatives. PLOS ONE. February 2014; 9(2):10.
21. Kelebek H, Canbas A, Jourdes M, Teissedre, P.L. HPLC-DAD-MS Determination of Colored and Colorless Phenolic Compounds in Kalecik Karasi Wines: Effect of Different Vineyard Locations. Anal Lett. 2011; 44(6):991-1008.
22. Wang Y, Liu Y, Xiao C, Liu L, Hao M, Wang J, Liu X. Simultaneous determination of 15 phenolic

constituents of Chinese black rice wine by HPLC-MS/MS with SPE. J Food Sci. 2014; 79(6):C1100-5.

23. Gouveia S, Castilho PC. Antioxidant potential of Artemisia argentea L'Hér alcoholic extract and its relation with the phenolic composition. Food Res Int. 2011; 44(6):1620-31.
24. Huang Y, Lu W, Chen B, Wu M, Li, S. Determination of 13 Phenolic Compounds in Rice Wine by High-Performance Liquid Chromatography. Food Anal Method. 2014; 8(4):825-32.
25. Salvatore E, Cocchi M, Marchetti A, Marini F, de Juan, A. Determination of phenolic compounds and authentication of PDO Lambrusco wines by HPLC-DAD and chemometric techniques. Anal Chim Acta. 2013; 761:34-45.
26. Euterpio MA, Pagano I, Piccinelli AL, Rastrelli L, Crescenzi C. Development and validation of a method for the determination of (E)-resveratrol and related phenolic compounds in beverages using molecularly imprinted solid phase extraction. J Agr Food Chem. 2013; 61(8):1640-5.
27. Dias F, Klassen A, Tavares MFM, David JM. Fast Determination of Phenolic Compounds in Brazilian Wines from Vale do São Francisco Region by CE. Chromatographia. 2013; 76(9-10):559-63.
28. Martelo-Vidal M.J, Vazquez M. Determination of polyphenolic compounds of red wines by UV-VIS-NIR spectroscopy and chemometrics tools. Food Chem. 158:28-34 (2014).
29. Medina MB. Determination of the total phenolics in juices and superfruits by a novel chemical method. J Funct Food. 2011; 3(2):79-87.
30. Chawla S, Rawal R, Kumar D, Pundir CS. Amperometric determination of total phenolic content in wine by laccase immobilized onto silver nanoparticles/zinc oxide nanoparticles modified gold electrode. Anal biochem. 2012; 430(1):16-23.
31. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A. 2003; 987:9.
32. Briscoe CJ, Stiles MR, Hage DS. System suitability in bioanalytical LC/MS/MS. J Pharm Biomed Anal. 2007; 44(2):484-91.