Development and Validation of a High-performance Liquid Chromatography–Diode-array Detection Method for the Determination of Eight Phenolic Constituents in Extracts of Different Wine Species

Amaç: Bu çalışmada amaç bazı yerel şarap ve meyve şarabı örneklerinde gallik asit, klorojenik asit, epigallocateşin, kafeik asit, vanilin, p-kumarik asit, rutin ve kersetin fenolik bileşiklerinin tayini için yeni bir HPLC-DAD yöntemi geliştirilmesi ve geçerli kılınmasını.

Gereç ve Yöntemler: Analizler, gradyan sistem kullanılarak bir Zorbax Eclipse C18 kolon (4.6 x 150 mm, 3.5-μm partikül boyutlu) üzerinde yürüütülmüştür. Akış hızının 1 mL/min olduğu sistemde hareketli faz A 10-mM fosforik asit ve hareketli faz B metanol olarak belirlenmiştir. Fenolik bileşikler, üç farklı dalga boyunda bir DAD kullanılarak izlenmiştir.

Bulgular: Geliştirilen ve geçerli kılınan yöntemin 1-100 ppm derişim aralığında doğrusal cevap verdiği gözlenmiştir. Geri kazanım değerleri %95-105 değerleri arasındadır ve tekrarlanabilir sonuçlardır. Geliştirilen yöntemi çeşitli şarap örneklerinde fenolik profili incelemek için uygulandı.

Sonuç: Yapılan çalışmanın sonucunda doğru, hassas ve güvenilir ölçüm yapan bir HPLC-DAD yöntemi geliştirilmiş ve şarap özütlerinde, şarabın antioksidan özellik göstermesini sağlayan fenolik maddelerin derişimlerini tayin etmek için başarıyla kullanılmıştır.

Anahtar kelimeler: Fenolik bileşikler, tayin, şarap, HPLC, validation

ABSTRACT

Objectives: A new HPLC method was developed and validated for the determination of some phenolic compounds; gallic acid, chlorogenic acid, epigallocatechin, caffeic acid, vanillin, p-coumaric acid, rutin, and quercetin in some local wine and fruit wine samples.

Materials and Methods: Analyses were performed on a Zorbax Eclipse C18 column (4.6 x 150 mm, 3.5-μm particle size) using a gradient system. Mobile phase A was a 10-mM phosphoric acid solution and mobile phase B was methanol using a flow rate of 1 mL/min. Phenolic components were monitored using a DAD at three different wavelengths.

Results: The developed and validated method was generally linear between the 1-100 ppm concentration range. Recovery values were obtained in the range of 95-105% and repetitive. The method was successfully applied to investigate the phenolic profiles of different wine samples.

Conclusion: As a result of the study, an accurate, sensitive and reliable HPLC-DAD method was developed. The method was successfully used to determine the concentrations of antioxidant phenolic constituents from some local wine extracts.

Key words: Phenolic compounds, determination, wine, HPLC, validation
INTRODUCTION

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

Winemaking is one of the most ancient of man’s technologies, known since the dawn of civilization, and has followed human and agricultural progress on the world. The earliest biomolecular archaeologic evidence for plant additives in fermented beverages dates from the early Neolithic period in China and Anatolia, when different types of fruits and cereals were used to make wine such as grapes, rice, millet, and fruits. 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. 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. Thus, winemaking releases benefical components such as phenolic compounds of antioxidant fruits besides grapes. There has been increasing interest in fruit wines produced from different types of fruit. 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.

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 geographic and environmental factors (soil type and climate). According to the literature, there are different methods to determine the phenolic contents of the different wine samples such as high-performance liquid chromatography–mass spectrometry (HPLC-MS), HPLC–diode-array detector (HPLC-DAD), gas chromatography (GC), capillary electrophoresis (CE), and spectrophotometric and electrochemical methods. These methods come with some advantages and disadvantages. Importantly, no studies have compared the phenolic profile of some local Turkish wines and fruit wines. In this study, a development and validation HPLC-DAD method is presented to evaluate the phenolic profile of some selected Anatolian wines and fruit wines.

MATERIALS 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 acquired from Merck (Darmstadt, Germany).

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

Calibration, linearity, and quality control (QC) samples

The eight analytes stock solutions were prepared by dissolving a weighed amount of the standard substance in ethanol at a 1 mg/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 the appropriate volume of stock solutions in volumetric flasks. Calibration curves were plotted, in triplicate, by analysing these freshly prepared standard solutions. Concentration values of the QC samples were as follows: the low-level concentration was 7.5 μg/mL, the medium-level concentration was 30 μg/mL, and the high level concentration was 80 μg/mL for each analyte.

Instruments and chromatographic conditions

Chromatographic analyses of phenolic compounds were performed using an Agilent 1260 HPLC system consisting of a quaternary pump model G1311B, an auto injector model G1329B, a thermostated column compartment model G1316A, and a DAD, model G4212B. The chromatograms were monitored and integrated by using Agilent ChemStation software. Chromatographic separations of the 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 according to their spectrums obtained from the Agilent ChemStation Software. Appropriate wavelengths were selected as: 214 nm for gallic acid, chlorogenic acid, and quercetin, 306 nm for vanillin, p-coumaric acid and rutin, and 333 nm for chlorogenic acid and caffeic acid. A gradient elution system was used to separate all analytes. For this purpose, two different mobile phases were used; Mobile phase A was 10 mM phosphoric acid solution and mobile phase B was methanol using a flow rate of 1 mL/min. The optimised gradient program 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 wines of Papazkarasi-type cultivar were purchased from local producers in Turkey. After removing the alcohol using a rotatory evaporator, the residual part of each
wine was lyophilized with a Christ Alpha 2-4 LD lyophilizator. The lyophilized extracts were dissolved in water at proper concentrations prior to experimentation.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

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

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

Method validation

System suitability test

Before performing any validation experiments, researchers should establish that the HPLC system procedure is capable of providing data of acceptable quality\(^\text{32}\) and make a 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.\(^\text{33}\) 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 to 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.\(^\text{32}\)

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

Calibration curves

Different concentration values of each phenolic compound were investigated to determine the dynamic range for the

| 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, theoretical 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 |
Limit of detection (LOD) and limit of quantification (LOQ) values of each substance were calculated by using calibration curve equations. As known, LOD is calculated by using the standard deviation (SD) of y-intercepts of regression lines. The sum of three times this SD value on the intercept of the calibration curve and intercept value 34 corresponds to the LOD signal value. In the same way, the sum of ten times this SD value on the intercept of calibration curve and intercept value corresponds to the LOQ signal value. Thus, LOD and LOQ values can be calculated using this approach. In this study, the limits of the developed method were determined using this calculation. The calibration curve dynamic ranges and related method limits are shown in Table 2.

**Accuracy**

Accuracy studies for the developed method were performed by analyzing samples of known concentration in triplicate at three different levels as low, medium, and high-level in the dynamic range. For this purpose, standard mixtures of each compound at three different concentration values were prepared by diluting the stock solution and the concentration values were 7.5, 30, and 80 μg/mL. After analyzing these standard solutions, the results obtained were investigated and the calculated concentration values were compared with known concentration values as recovery. This comparison was made both for intra-day studies and inter-day studies. The results are presented in Table 3.

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

**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 SD (RSD) for a statistically significant number of samples. Table 3 also shows the precision of the method through the presentation of RSD values obtained from three repeated analyses of known amounts of standard at three different levels. For most of the components, these RSD values for intra-day studies were lower than 1%, which shows that the method was very precise in intra-day studies, with the exception of 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 these three substances should be analyzed using a daily calibration system. Unfortunately, the method developed cannot be precise for inter-day studies and analysts should work carefully and when preparing standard solutions, low-concentration values need particular attention.

**Specificity**

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

**Robustness and ruggedness**

The robustness and ruggedness of the method were investigated by deliberately changing some analytic parameters in the range of ±10%. The investigated parameters were injection volume, temperature, and concentration of phosphoric acid. Injection volume and temperature were parameters related with instrumentation 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 be seen that the recovery values were appropriate to the 85-115 percentage rule. Especially at low concentration level, recovery values were affected by the changes. This means that if the analyte amount in the sample was at low level, the analyst should be more careful on analysis. The obtained recovery values were in the range between 88-105%, which shows that this method is robust.

**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. Table 5 shows the results for this analysis.

### 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 |

LOD: Limit of detection, LOQ: Limit of quantification
When the analysis results were investigated, it was seen that epigallocatechin could not be detected in these wine samples. If a comparison between the other phenolic compounds found in these wine samples is needed, it can be understood that black mulberry contains phenolic compounds, more than in 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 black mulberry wine had higher TPC and TOAC properties than other wine samples. Analysis results of the wine samples support these TPC and TOAC test results.

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

| Analyte            | Concentration level | Intra-day variation | Inter-day variation |
|--------------------|---------------------|---------------------|---------------------|
|                    |                     | Accuracy (mg/mL)    | RSD (%)             | Accuracy (mg/mL) | 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              | 0.09                | 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); RSD: Relative standard deviation

Figure 2. A sample HPLC chromatogram of 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 could not be detected in these wine samples. If a comparison between the other phenolic compounds found in these wine samples is needed, it can be understood that black mulberry contains phenolic compounds, more than in 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 black mulberry wine had higher TPC and TOAC properties than other wine samples. Analysis results of the wine samples support these TPC and TOAC test results.
Table 4. Obtained recovery values during robustness-ruggedness studies. Results are expressed as the mean of triplicates ± SD

| Analyte          | Injection volume | Concentration of phosphoric acid | Temperature | Analyte | Concentration of phosphoric acid | Temperature | Analyte | Concentration of phosphoric acid | Temperature |
|------------------|------------------|----------------------------------|-------------|---------|----------------------------------|-------------|---------|----------------------------------|-------------|
|                  | 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 acid | 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 |
|                  | 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     | 100.03±0.28 | 99.54±0.41 | 99.52±0.06 | 99.10±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 |

L: Low level, M: Medium level, H: High level

Table 5. Phenolic composition of the wine extracts using the developed method. Results are expressed as the mean of triplicates ± SD and as µg/mg sample

| Analyte          | Blueberry wine | Black mulberry wine | Cherry wine | Papazkarasi wine |
|------------------|----------------|---------------------|-------------|------------------|
| Gallic acid      | 1.2±0.070      | 1.66±0.085          | 0.73±0.014  | 0.20±0.028       |
| Chlorogenic acid | nd             | 1.56±0.096          | nd          | nd               |
| Epigallocatechin | nd             | nd                  | nd          | nd               |
| 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       |

nd: Not detected
CONCLUSION
This developed and validated method was applied successfully to
determine the phenolic constituents of different wine samples.
Our results were in good agreement with TPC and TOAC tests
published previously. The method can also be used for the
determination of the phenolic compounds of styrrax liquids and
different pekmez samples.

Conflict of Interest: No conflict of interest was declared by the
authors.

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