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Determination of Some Phenolic Compounds from Commercial Wine Vinegar Samples in Turkey by High Performance Liquid Chromatography

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ABSTRACT: Seven different commercial wine vinegars from Turkey were analyzed to verify the presence of phenolic compounds. A sensitive, simple and accurate method was developed to determine eight bioactive phenolic compounds (chlorogenic acid, caffeic acid, *p*-coumeric acid, rutin, ferullic acid, quercetin, naringenin, kaempferol). Quantification and identification of phenolic compounds was carried out by a validated HPLC–UV method. The analytical parameters were evaluated, establishing limit of detections, LODs (from 0.01 to 0.02 mgL^{-1}) and limit of quantifications, LOQs (from 0.03 to 0.06 mgL^{-1}) in the present method.The correlation coefficients were all above 0.998. The method was validated for specificity, linearity and precision.

Keywords *–Wine vinegar, phenolic compounds, HPLC*

1. Introduction

Phenolic compounds, mostly isolated from plants are secondary metabolites that reveal a broad spectrum of biological activities (Demirtas et al., 2013; Elmastas et al., 2015; Elmastas et al., 2016; Erenler et al., 2014; Erenler et al., 2016; Erenler et al., 2017). Grape (*Vitis vinifera*) is one of the largest fruit crops in Turkey (especially Tokat, Manisa, Erzincan region). Most of the grape has been processed to different products such as raisin, vine, vinegar, grape juices in the food industry. Wine Vinegar is a liquid largely produced in Central European and Mediterranean countries such as Turkey which give rise to products of greatly differing quality. Also it is so usual food product widely available in the market.

Vinegar has two main biotechnological processes. One of them alcoholic fermentation (using yeast, e.g., *Saccharomyces cerevisiae*) the other one is acetous fermentation (using acetic acid bacteria) (Budak et al., 2014). In Turkey traditionally vinegar is made from raw plant materials, for example,grains, grapesapples(Junior et al., 2014).

Wine vinegar has different kinds of beneficial effects to consumers, such as antidiabetic, antioxidative, antiobesity, antihypertensive, antimutagenic, anti-lipid peroxidation, anticancerand antimicrobial effects (Laranjinha et al., 1994; Salbe et al., 2009). Besides direct consumption, vinegar plays an important role infood production (sauces, ketchups and mayonnaise).

Eventhough HPLC-UV detector is widely available and easy to use, still there are not many literatures on phenolic compounds of wine vinegar by this method. The present study was to determine and identificationthe substance of phenolic compounds in wine vinegar commercially available from market.

2. Material and Methods

2.1. Chemicals and Materials

Analytical grade reagents and chemicals were used in the development of this study and kaempferol, caffeic acid, ferullic acid, quercetin, rutin, *p*-coumaricacid, chlorogenic acid, naringeninwere obtained from the Sigma-Aldrich Co. (Sternheim, Germany). Acetonitrile formic acid and methanol were obtained from Merck Co. (Istanbul, Turkey).HPLC-grade waterwas used from a Milli-Q water purification system (Millipore, Bedford, USA).Stock solutions of each compound were prepared in methanol.

2.2. Standard Solutions

Stock standard solutions (1000 μg mL^{-1}) were prepared by separately dissolving the accurate amount of each standard into the HPLC grade methanol and stored refrigerator at -20°C. Working standard solutions, that contain all the phenolics were prepared diluting stock standard solutions. The standard solutions were sonicated after preparation and before using (injection in the HPLC) in order to ensure maximal solubilisation of each phenolic compound in the mixture.

2.3. Samples

Seven different brand wine vinegars were sampled from the markets and used to test of the proposed method. Before analysis all of the samples were filtered 0.45 m PTFE cartridges (Sartorius AG, Goettingen, Germany).

2.4. Optimization of Chromatographic Conditions

The quantitative analysis of the phenolic compounds was performed on a Perkin Elmer Series 200 HPLC. As stationary phase a PhenomenexKromasil C18 (4×20 cm, 5 μm) was used. A gradient of solvent A:acetonitrile and solvent B:deionizedwater: formic acid (90:5) was applied as follows: 0–5 min, 5% solvent A; 5–20 min, 22% solvent A; 20–40 min, 95% solvent A and 40-45 min, 100% solvent B to wash and equilibrate of the column. Other parameters: flow rate 1 $mLmin^{-1}$, injection volume: 20 μL, UV detector, detection wavelength 280 nm; column temperature: 30 °C, stop time 40 min. Three replicates are carried out for each sample. Identification and qualitative analyses were performed at each retention time by comparison with standard spectra. The amounts of phenolic compounds were calculated using a calibration curve equation for standards. The results were expressed in milligram per liter (Erenler et al., 2015).

2.4.1. Validation of Chromatographic Conditions

All samples and standards were analyzed in triplicate and precision was evaluated by six replicated injections of the standard solutions. The analytical method for the quantification of chlorogenic acid, caffeic acid, *p*-coumeric acid, rutin, ferullic acid, quercetin, naringenin, kaempferolwerevalidated by HPLC, according to the International Conference

on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH, 2005) and Brazilian regulation of the National Health Surveillance Agency (Anvisa, 2003).

Specificity is the ability of the method to accurately measure the analyte response in the presence of all interferences. The linearity was determined based on the calibration curve obtained for each phenolic compound separately. The slope, intercept, as well as the coefficient of determination (R^2) were calculated for each calibration curve. The limit of detections (LODs) and the limit of quantifications (LOQs) of the developed method were calculated from the linearity curve, using the equations: LOD = 3.3S/SD and LOQ = 10S/SD, respectively. The accuracy of the method was measured through a recovery assay. The study was performed after three replicate. The accuracy was expressed as a percentage of the amount recovered compared with the standard concentrations.

The repeatability was investigated using three replicate injections The results are expressed as the relative standard deviation percentage of the measurements (RSD %).

3. Results and Discussion

The quantification of phenolic compounds by HPLC was carried out by using calibration curves of reference compounds (Fig.1). To develop the method and identify peaks (chlorogenic acid, caffeic acid, *p*-coumeric acid, rutin, ferullic acid, quercetin, naringenin, kaempferol),standard stock solution (1000 μg mL^{-1}) wasprepared. Five dilute solutions from these stocks were used for calibration curves of each standard. 20 μL samples were injected into the HPLC system (Fig.2).After validation, HPLC method was successfully applied to wine vinegar samples. The method provided low LODs and LOQs for eight phenolic compounds. All results in the calibration curves showed good linearity within the indicated concentration ranges, with R^2 higher than 0.998.

The vinegar included the most chlorogenic acid among the tested compounds with the 5.40-33.76 mg/mL.Ferulic acid was the second abundant compound in vinegar with the 0.35-3.10 mg/mL. The other compounds were found in vinegar at various quantities. The chlorogenic acid is significant phenolic compound having a large variability of biological and pharmaceutical properties including antioxidant, anti-inflammatory.

Chlorogenic acid plays an important role on glucose and lipid metabolism regulating diabetes, cardiovascular disease, obesity, cancer and hepatic steadies. A great many health benefits of chlorogenic acid may provide for treatment and prevention of some chronic diseases. Therefore, usage of vinegar in daily food could be essential for health (Tajik et al., 2017).

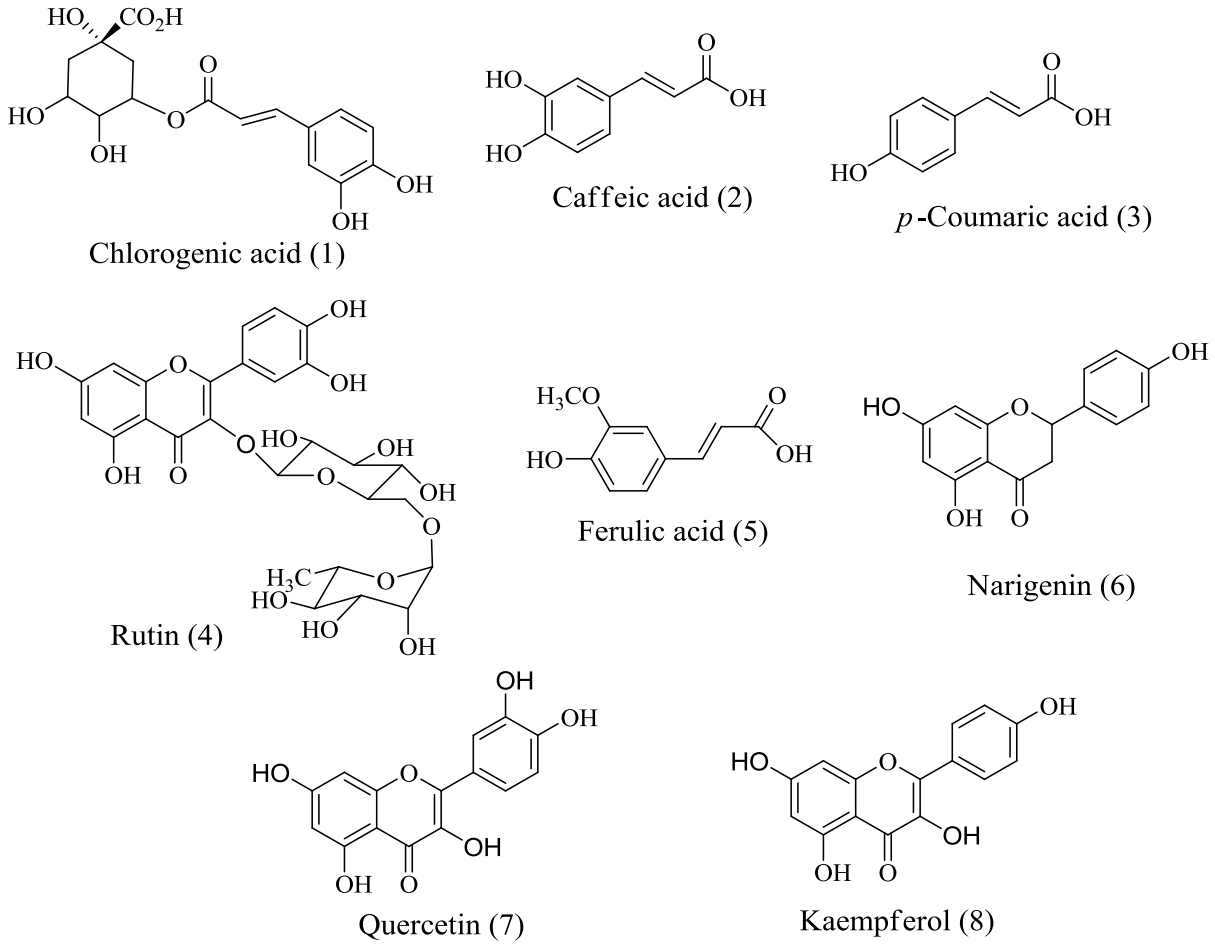

Figure 1. *Structures of Phenolic compounds*

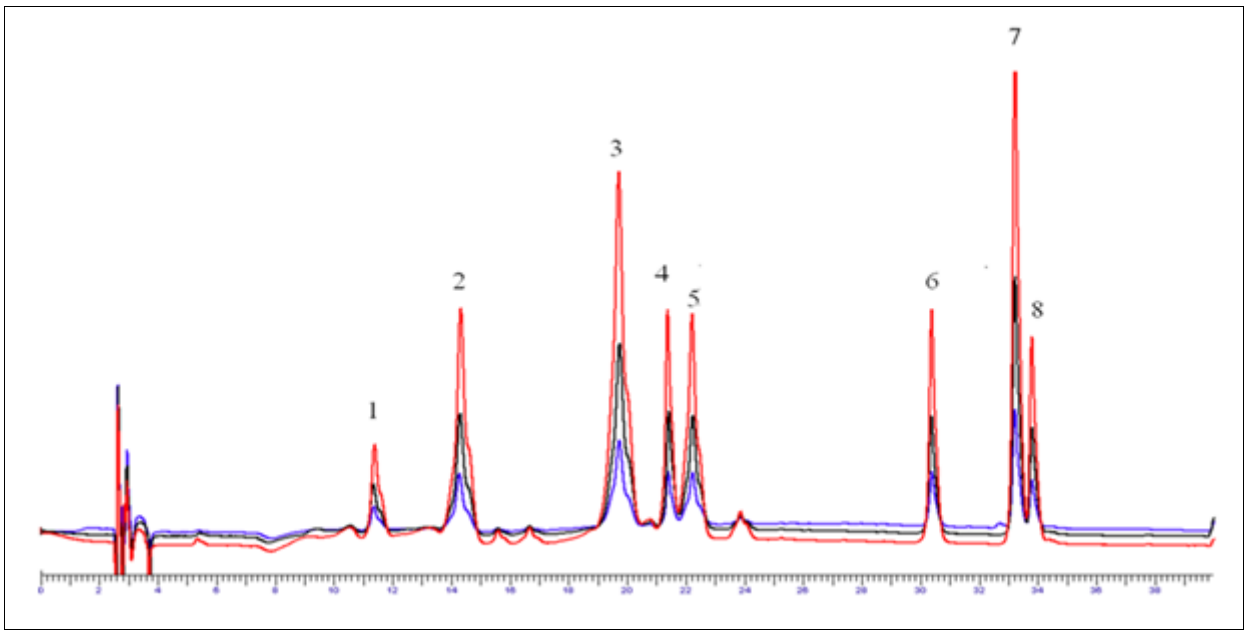

Figure 2. *HPLC Chromatogram of phenolic standards; chlorogenic acid (1), caffeic acid (2), p-coumaricacid (3), rutin (4), ferullic acid (5), quercetin (6), naringenin (7), kaempferol (8)*

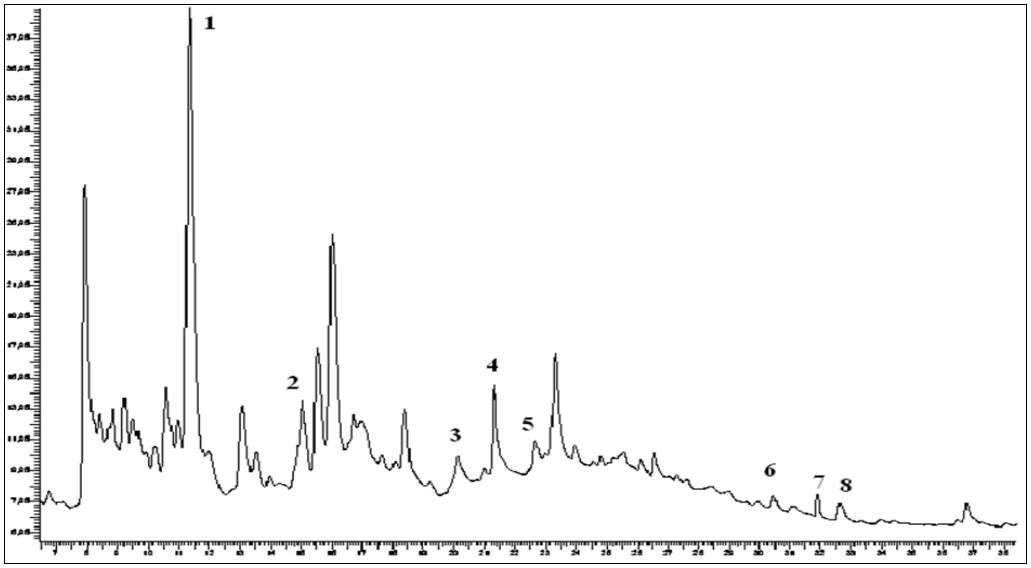

Figure 3. *HPLC chromatogram of the vinegar samples; chlorogenic acid (1), caffeic acid (2), p-coumaric acid (3), rutin (4), ferullic acid (5), quercetin (6), naringenin (7), kaempferol (8)*

Table 1. *HPLC Method Validation*

Compound	Range (mg/L)	RT (min)	R^2 değeri	Linear Range (mg/L)	LOD	LOQ
1. Chlorogenic acid	5.40-33.76	11,35	0,999	0.05-50	0.02	0.06
2. Caffeic acid	0.25-2.20	14,31	0,999	0.025-25	0.013	0.04
3. *p*-Coumaric acid	0.24-1.55	19,74	0,999	0.025-25	0.013	0.04
4. Rutin	0.4-17.25	21,42	0,999	0.05-50	0.015	0.04
5. Ferulic Acid	0.35-3.10	22,23	0,999	0.025-25	0.02	0.06
6. Quercetin	0.20-0.35	30,37	0,999	0.025-25	0.015	0.04
7. Naringenin	0.16-0.17	33,23	0,999	0.025-25	0.01	0.03
8. Kaempferol	0.25-0.35	33,80	0,998	0.025-25	0.015	0.04

A total of 8 phenolic compounds were quantified in wine vinegar including 1 flavonoid (naringenin) 3 flavonols (kaempferol,rutin and quercetin) and 4-hydroxycinnamic acids (chlorogenic, caffeic, *p*-coumaric and ferulic acids).

4. Conclusion

A simple, rapid, and reliable method was developed and validated for the determination of vinegar samples by HPLC. Satisfactory method validation results were afforded after

optimization of the experimental conditions. The measurement uncertainty of the entire procedure was also calculated.

The present was studied with the samples commercially obtained seven different brand vinegar in Turkey. A simple and effective HPLC method was developed for simultaneous determination and quantification of phenolic compounds and found to be accurate, precise and linear throughout the analytical range.

The selection of the HPLC conditions was guided by the requirement for obtaining chromatograms with better resolution of adjacent peaks within short time. The method was specific in analyzing vinegar samples. The method may be used to review the quality of commercially available vinegars.

HPLC analysis of vinegar samples depicted the presence of polyphenolics such as namelychlorogenic acid, caffeic acid, *p*-coumeric acid, rutin, ferullic acid, quercetin, naringenin, kaempferol.Our data showed that high amount of chlorogenic acid were found in wine vinegar samples. Chlorogenic acids reduce blood pressure in spontaneously hypertensive rats and humans.

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