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Simultaneous analysis of 25 phenolic compounds in grape juice for HPLC: Method validation and characterization of São Francisco Valley samples

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ABSTRACT

A HPLC method for simultaneous determination of 25 phenolic compounds in grape juice was optimized, validated and applied in the characterization of juices produced in São Francisco Valley (SFV), Brazil. The performance characteristics of the method were established by assays with standard solutions of phenolic compounds, spiked and unspiked samples. Linearity, matrix effects, trueness, precision, detection and quantification limits were evaluated. Linearity was demonstrated in the concentration ranges tested for all phenolic compounds. Significant matrix effects were not identified for the studied compounds. Mean recoveries ranged from 86.18 to 106.50%, demonstrating no lack of trueness. Precision of the method was confirmed for the 25 phenolic compounds, with acceptable repeatability relative standard deviations (from 0.71 to 9.24%) and within-reproducibility relative standard deviations (from 1.34 to 9.26%) for unspiked and spiked samples. The theoretical limits of detection and quantification of the method varied from 0.001 to 0.19 μg mL^{-1} and 0.003 to 0.37 μg mL^{-1}, respectively. The results of the validation process showed that the proposed method is fitness for purpose. This method was able to identify simultaneously 25 phenolic compounds and had advantages such as low consumption of solvents and easy sample preparation. The phenolic profile of the grape juices from SFV varied according the grape cultivar. Phenolics of the anthocyanins and tannins class predominated in red grape juices, while in white grape juice phenolic acids and tannins were found at high concentrations.

1. Introduction

Grape juice is reported as an excellent alternative of non-alcoholic beverage rich in antioxidant substances, mainly phenolic compounds [1,2]. Consumption of grape juice is associated with several health benefits, such as: increase of antioxidant capacity, improvement of the endothelial function, inhibition of platelet aggregation, decrease of plasma protein oxidation, reduction of the low-density lipoprotein (LDL) oxidation and improvement of cardiovascular and neurocognitive function [3–5].

In addition to the functional properties, the phenolic compounds present in grape juice still contribute to the definition of the sensory characteristics of this product [6]. Biotic and abiotic factors, such as climatic conditions, sunlight exposure and hydric status [7,8]. Each grape cultivar shows a peculiar phenolic composition and the evaluation of this profile is also suggested as a tool for authenticity and identification of grape beverages [9].

The terrain and climate characteristics become important in Brazilian viticulture context, once the main winegrowing regions, southern and São Francisco Valley (SFV) are very different. The SFV is located in the Northeast of Brazil and characterized by a tropical semi-arid climate with high sunlight exposure during most of the year [10]. Therefore, the characterization of grape juice produced in this region is a research field still unexplored, once there are no reports about the typicality and the phenolic composition of these products.

Currently, there are available several methodologies for identification and quantification of phenolic compounds in grape juice. In assays with purpose of total phenolics and anthocyanins determination, classical spectrophotometric methods are commonly used, such as Folin–Denis and differential pH, respectively [11]. However, these methods are not specific for grape juice matrix and frequently offer overestimate phenolic content due to the lack of selectivity [12].

For the identification and quantification of phenolic compounds purpose, chromatographic techniques are recommended and widely used for grape juice [13–19]. Nevertheless, the election of an appropriate methodology to perform these analyses is still a challenge, due to the diversity of available protocols and the absence of fully validated methods

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for simultaneous determination of different phenolic classes in grape juice matrix.

Few studies provided data related to the validation of chromatographic methods for the related scope [20–22]. Generally, the validation strategies do not address all performance parameters necessary to evaluate the fitness for purpose [23]. Moreover, in many studies the more frequently investigated parameters were recovery and limits, which shows the fragility of the proposed methodologies.

Then, this study presents: i) a fully validated method for simultaneous determination of 25 phenolic compounds, which belong to the classes of anthocyanins, flavonols, phenolic acids and tannins, in grape juice; and ii) the application of the validated method to the characterization of grape juices produced in SFV, Brazil.

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile and phosphoric acid 85% HPLC grade were supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil), J. T. Baker (Phillipsburg, NJ, USA) and Fluka (Switzerland), respectively. Ultrapure water was obtained from a Purelab Option Q Elga System (USA).

2.2. Standards

Caffeic acid, cinnamic acid and gallic acid standards were purchased from Chem Service (West Chester, USA). Kaempferol-3-O-glucoside, pelargonidin-3-O-glucoside chloride (callistephin chloride), (+)-catechin, cyanidin-3,5-diglucoside-chloride (cyanin chloride), cyanidin-3-glucoside-chloride (kuromanin chloride), (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, isorhamnetin-3-O-glucoside, malvidin-3,5-di-O-glucoside-chloride (malvin chloride), myricetin, delphinidin-3-glucoside-chloride (myrtillin chloride), malvidin-3-glucoside-chloride (oenin chloride), peonidin-3-O-glucoside chloride, procyanidin A2, procyanidin B1, procyanidin B2, quercetin (dihydrate), resveratrol and rutin standards were obtained from Extrasynthese (Genay, France). Chlorogenic acid and *p*-coumaric acid were purchased from Sigma (United Kingdom). Stock solutions of each standard were prepared in methanol. A pool intermediate solution with the 25 studied phenolics was prepared by dilution of the respective stock solutions in 0.85% phosphoric acid solution (Table 1).

2.3. Samples

Grape juices were elaborated on Enology Laboratory of the Embrapa Semiárido (Petrolina — PE, Brazil). Six varieties of the red grapes and one variety of white grape grown in SFV were employed for the production of varietal grapes juices. The red grapes used were: Isabel Precoce (*Vitis labrusca*); BRS Cora and BRS Violeta (hybrid grapes); Tempranillo, Syrah and Alicante Bouschet (*Vitis vinifera* L.). The Moscato Canneli (*V. vinifera* L.) was the white grape used in the elaboration of juice.

After the harvest, grapes were maintained in cold chamber at 10 °C ± 2 °C during 12 h. Then, the grapes were sanitized with sodium hypochlorite solution at 200 mg L^{-1} and berries were manually destemmed. The elaboration of the grape juices was carried out at 75 °C ± 5 °C, during 1 h, using an artisanal equipment for water vapor extraction [24]. To each litter of grape juice, 0.8 g of potassium metabisulphite (Synth, Diadema, Brazil) was added. The grape juices were stored in the cellar of the Enology Laboratory at 18 °C ± 2 °C.

Table 1
Concentration of each stock and intermediate solution of the 25 studied phenolic compounds.

Classification	Phenolic	Stock solution (μg mL^{-1})	Intermediate solution (μg mL^{-1})
Anthocyanins	Cyanidin-3,5-diglucoside-chloride	2500	100.00
	Cyanidin-3-glucoside-chloride	2500	100.00
	Delphinidin-3-glucoside-chloride	2500	100.00
	Malvidin-3,5-di-O-glucoside-chloride	2500	100.00
	Malvidin-3-glucoside-chloride	2500	50.00
	Pelargonidin-3-O-glucoside-chloride	2500	25.00
	Peonidin-3-O-gluoside-chloride	500	6.25
Flavonols	Isorhamnetin-3-O-glucoside	2500	12.50
	Kaempferol-3-O-glucoside	2500	12.50
	Myricetin	5000	12.50
	Quercetin	1000	12.50
	Resveratrol	1000	25.00
	Rutin	1000	12.50
Phenolic acids	Caffeic acid	2000	50.00
	Chlorogenic acid	1000	50.00
	Cinnamic acid	2000	50.00
	Gallic acid	2500	25.00
	p-Coumaric acid	1000	50.00
Tannins	(−)-Epicatechin	5000	50.00
	(−)-Epicatechin gallate	5000	25.00
	(−)-Epigallocatechin gallate	5000	25.00
	(+)-Catechin	5000	50.00
	Procyanidin A2	2500	25.00
	Procyanidin B1	500	6.25
	Procyanidin B2	2500	50.00

For the in-house validation experiments, the Isabel Precoce juice was selected as a representative matrix. First, it is due to the fact that this is the mainly variety grown in Brazil for the juice production, and second, because it represents a red and complex matrix.

2.4. Equipment

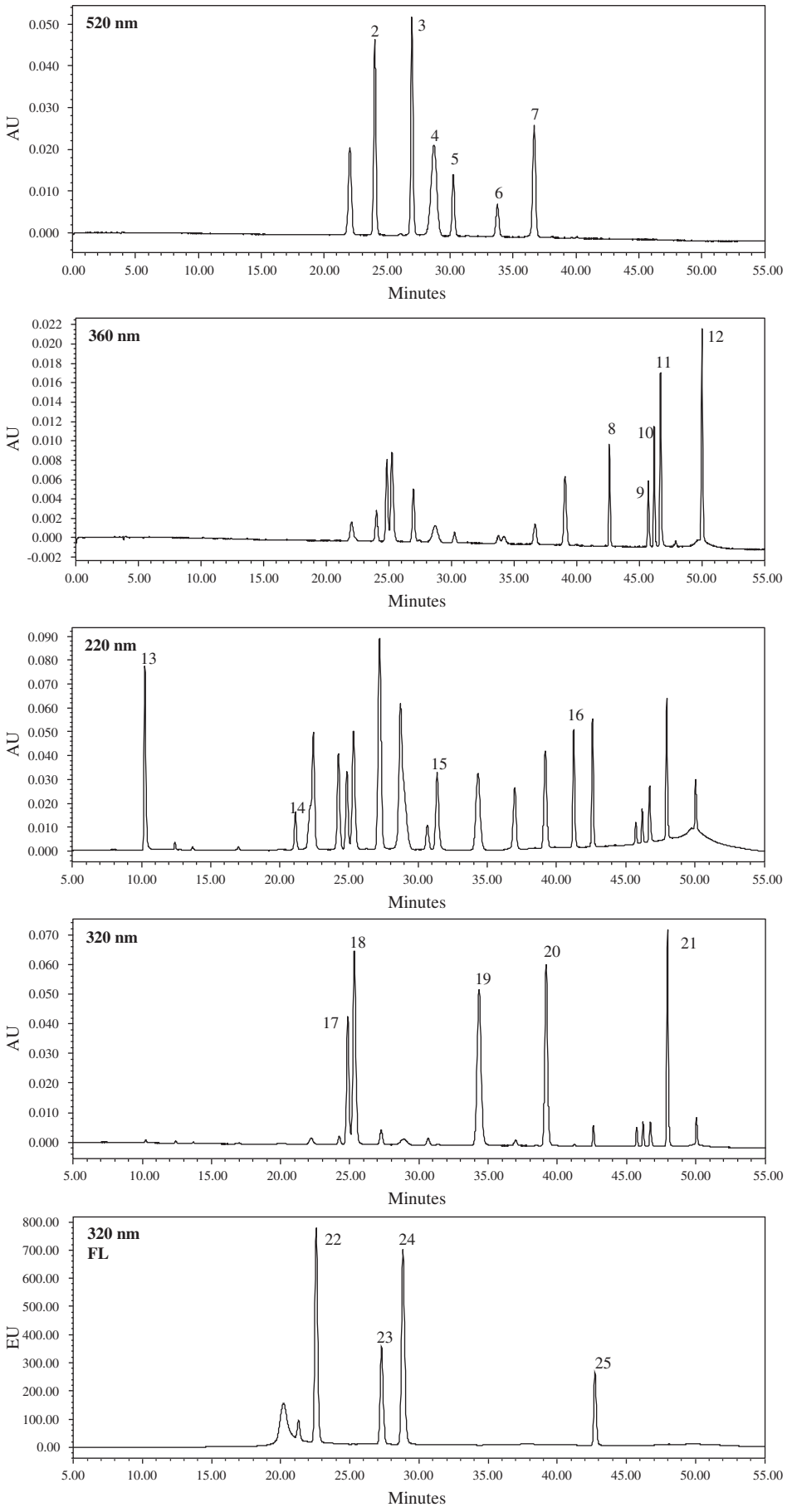
The analyses were performed using a HPLC system Waters e2695 Separation Module Alliance equipped with a quaternary solvent pump and an automatic injector. For the phenolic determination, a diode array detector Waters model 2998 and a fluorescence detector Waters model 2475 were employed. Data acquisition and processing were carried out using the Waters Empower™ 2 software (Milford, USA).

2.5. Analytical procedure

The method previously described by Corrêa et al. [25] had the chromatographic conditions optimized for the grape juice matrix. An aliquot of 500 μL of the grape juice was diluted to 1 mL with 0.85% phosphoric acid solution and filtered through a 0.45 μm nylon membrane (Allcrom—Phenomenex, USA). The injection volume was 10 μL. The Gemini NX C-18 column (150 mm × 4.6 mm × 3 μm) (Phenomenex, USA) was maintained at 40 °C. The mobile phase consisted of a gradient mixture of a solvent A (0.85% phosphoric acid solution) and solvent B (acetonitrile), with a flow-rate of 0.5 mL min^{-1}. The gradient was started with 100% of solvent A and adjusted for 93% of solvent A and 7% of solvent B in 10 min; 90% of solvent A and 10% of solvent B in 20 min; 88% of solvent A and 12% of solvent B in 30 min; 77% of solvent A and 33% of solvent B in 40 min; 65% of solvent A and 35% of solvent B in 45 min; and 100% of solvent B in 55 min.

The standard solutions were injected for identification of the wavelengths in which occurred the absorption of the compounds and their

Fig. 1. Chromatograms of the phenolic compounds and their respective retention time (RT). 1: cyanidin-3,5-diglucoside-chloride (RT: 21.8 min); 2: delphinidin-3-glucoside-chloride (RT: 23.8 min); 3: malvidin-3,5-di-o-glucoside-chloride (RT: 26.6 min); 4: cyanidin-3-glucoside-chloride (RT: 28.4 min); 5: pelargonidin-3-o-glucoside-chloride (RT: 29.7 min); 6: peonidin-3-o-glucoside-chloride (RT: 33.4 min); 7: malvidin-3-glucoside-chloride (RT: 36.3 min); 8: rutin (RT: 42.7 min); 9: kaempferol-3-O-glucoside (RT: 45.6 min); 10: isorhamnetin-3-O-glucoside (RT: 46.1 min); 11: myricetin (RT: 46.4 min); 12: quercetin (49.8 min); 13: gallic acid (RT: 10.0 min); 14: procyanidin B1 (RT: 20.8 min); 15: (−)-epigallocatechin gallate (RT: 30.8 min); 16: (−)-epicatechin gallate (RT: 40.9 min); 17: chlorogenic acid (RT: 24.3 min); 18: caffeic acid (RT: 25.0 min); 19: *p*-coumaric acid (RT: 33.7 min); 20: cinnamic acid (RT: 38.5 min); 21: resveratrol (RT: 47.7 min); 22: (+)-catechin (RT: 22.3 min); 23: procyanidin B2 (RT: 27.0 min); 24: (−)-epicatechin (RT: 47.7 min); 25: procyanidin A2 (RT: 49.8 min).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respective retention times. Fluorescence detector was used at 320 nm emission for identification of the following tannins: (+)-catechin, procyanidin B2, procyanidin A2 and (−)-epicatechin. The diode array detector was employed in four wavelengths, being: 220 nm for identification of the gallic acid and the tannins: (−)-epigallocatechin gallate, (−)-epicatechin gallate and procyanidin B1; 320 nm for resveratrol and phenolics acids; 360 nm for flavonols and 520 nm for anthocyanins.

The chromatograms obtained for the standard solutions of the 25 studied phenolic compounds and their respective retention time are presented in Fig. 1.

2.6. In-house validation procedure

The performance characteristics of the method were established by assays employing standards solutions, sample blanks and spiked samples. Linearity, matrix effects, trueness (recovery), precision under repeatability and within-reproducibility conditions, theoretical limits of detection and quantification were the parameters evaluated [23]. The significance level adopted for the statistical analysis was $\alpha = 0.05$.

2.6.1. Linearity

The linearity was evaluated as describe by Souza and Junqueira [26]. Calibration curve was prepared by dilution of the pool intermediate solution in 0.85% phosphoric acid solution, obtaining six evenly spaced concentration levels. For each level, two independent replicates were prepared and injected in a random order. Four concentration ranges were defined according to the analyte concentrations commonly found in grape juice, as described in Table 2. Blanks were also prepared, in duplicate, for evaluation of the noise, but not included in regression analysis.

The estimation of the regression parameters was done by the ordinary least squares method (OLSM). The residual plots were examined for obvious patterns. The outliers were indicated by points out of the range $\pm\, t_{(0.975;n-2)}s_{res}$, being the s_{res} the standard deviation of the regression residual. Outliers were treated by Jacknife standardized residuals test. This test was applied successively until no further outliers were detected, allowing a maximum exclusion of 22.2% in the original number of results. The assumptions of linear regression were checked by the following tests: residual normality by Ryan and Joiner test, independence by Durbin and Watson test, and homoscedasticity by modified Levene test. *F-tests* were performed to evaluate the adjustment to the linear model by evaluation of regression and lack of fit significances.

2.6.2. Matrix effects

Matrix effects were evaluated by applying the method of standard additions. Two calibration curves were prepared (solvent and matrix matched curves) with the same analyte concentration levels. Each level was prepared in two independent replicates, which were analyzed in a random order. The solvent curve was prepared as described for the linearity assessment. To prepare the matrix matched curve 500 μL of the Isabel Precoce juice, previously filtered through a 0.45 μm nylon membrane, were added with 50, 70, 90, 110, 130 and 150 μL of the pool intermediate solution. The volume was completed to 1.0 mL with 0.85% phosphoric acid solution. Blanks were prepared in duplicate for the evaluation of the noise, but not included in regression analysis.

OLSM was used to calculate the values of the slope, intercept and variances for the solvent and matrix matched curves, with a previously check of the assumptions. The slopes of both curves were compared by t-test [27]. The interceptions were not compared due to the fact that the samples had a native concentration of the studied phenolics.

2.6.3. Trueness (recovery) and precision

Unspiked and spiked samples of Isabel Precoce grape juice were prepared in twelve independent replicates. To each concentration level, the replicates were split in 4 analytical batches, which were evaluated by two different analysts.

The native concentrations of each phenolic compound in the grape juice samples, i.e., the obtained results for unspiked samples, were considered for the definition of the spiking levels, avoiding final concentrations out of the linear range.

Recovery and precision were studied as described by Souza et al. [27]. Outliers were investigated by the application of the Grubbs test. Lack of trueness was evaluated by the mean recovery, considering the European Commission criteria [28], that established a satisfactory range between 80 and 110% for mass fractions $\geq 10\ \mu g\ kg^{-1}$. Precision was investigated by assays with unspiked and spiked samples. The relative standard deviation under repeatability (RSD_r) e within-reproducibility (RSD_R) conditions was estimated by ANOVA for each concentration level. The assumptions related to ANOVA were previously checked: residual normality by Ryan and Joiner test and homoscedasticity by modified Levene test. Acceptable RSD_R was established by Horwitz equation. The RSD_r was considered acceptable when falling within two thirds of the value estimated by the Horwitz equation.

2.6.4. Limits of detection and quantification

Due to the unavailability of grape juice free of the studied phenolics, the theoretical limits were stated by the analysis of solvent blank in seven replicates. The limits of detection and quantification were estimated as the mean plus three and ten standard deviation of the results, respectively.

2.7. Characterization of grape juices

The validated method was employed for the determination of the phenolic profile of six red grape juice samples (Isabel Precoce, BRS Cora, BRS Violeta, Tempranillo, Syrah, Alicante Bouschet) and one white grape juice sample (Moscato Canelli), produced in SFV, in 2012. Three batches of each grape juice sample were analyzed in triplicate. Additional dilutions were made for the samples in which the phenolic concentrations were above the validated work range. ANOVA and Tukey test were performed at the 0.05 significance level.

3. Results and discussions

3.1. Linearity

The results obtained for the linearity assessment are shown in Table 3.

Outliers were detected by Jacknife standardized residual test for (−)-epicatechin gallate, (−)-epigallocatechin gallate, caffeic acid, chlorogenic acid, cinnamic acid, gallic acid, isorhamnetin-3-O-glucoside, myricetin, p-coumaric acid, peonidin-3-O-glucoside chloride, procyanidin B1 and quercetin. Considering the calibration curves of the other phenolic compounds outliers were not identified. The Ryan-Joiner test indicated that residuals are normally distributed ($p > 0.01$ for (+)-catechin; $p > 0.05$ for (−)-epigallocatechin gallate and pelargonidin-3-O-glucoside chloride; $p > 0.10$ for the other phenolic compounds). Independency was demonstrated by Durbin–Watson test ($p > 0.025$) for the studied compounds although inconclusive results were observed for caffeic acid, cinnamic acid, isorhamnetin-3-O-glucoside, kaempferol-3-O-glucoside and procyanidin B1 ($p < 0.01$). The Levene t-statistic indicated that the residuals were homoscedastic for all phenolics ($p > 0.05$). In all cases, the regression was significant ($p < 0.001$) and lack-of-fit was not observed ($p > 0.05$), confirming linearity in the studied concentration ranges.

Linearity represents an important performance parameter addressed in validation studies. Fracassetti et al. [29] validated a method for determination of catechin and caffeic acid in white grape juice by ultra-performance liquid chromatography and prepared calibration curves in the ranges from 0.5 to 80 $\mu g\ mL^{-1}$ and from 0.5 to 50 $\mu g\ mL^{-1}$, respectively. A HPLC-DAD method for the quantification of four flavan-3-ols and five anthocyanins in skin and seed extracts of red grape varieties

Table 2
Distribution of phenolic compounds according to the concentration range in calibration curve and respective corresponding range in grape juice.

Phenolic compounds	Concentration range (μg mL^{-1})	
	Calibration curve	Corresponding in grape juice
Rutin, kaempferol-3-O-glucoside, isorhamnetin-3-O-glucoside, myricetin, quercetin, peonidin-3-O-glucoside chloride and procyanidin B1	0.625, 0.875, 1.125, 1.375, 1.625 and 1.875	1.25, 1.75, 2.25, 2.75, 3.25 and 3.75
Procyanidin A2, resveratrol, pelargonidin-3-O-glucoside chloride, gallic acid, (−)-epigallocatechin gallate, (−)-epicatechin gallate.	1.25, 1.75, 2.25, 2.75, 3.25 and 3.75	2.50, 3.50, 4.50, 5.50, 6.50 and 7.50
(+)-Catechin, procyanidin B2, (−)-epicatechin, chlorogenic acid, caffeic acid, *p*-coumaric acid, cinnamic acid and malvidin-3-glucoside-chloride	2.50, 3.50, 4.50, 5.50, 6.50 and 7.50	5.00, 7.00, 9.00, 11.00, 13.00 and 15.00
Cyanidin-3,5-diglucoside-chloride, delphinidin-3-glucoside-chloride, malvidin-3,5-di-O-glucoside-chloride and cyanidin-3-glucoside-chloride	5.00, 7.00, 9.00, 11.00, 13.00 and 15.00	10.00, 14.00, 18.00, 22.00, 26.00 and 30.00

was validated by Munõz et al. [18], considering ranges from 10 to 400 μg mL^{-1} and from 1 to 250 μg mL^{-1}, respectively, for the calibration curves. Dias et al. [30] carried out the optimization and validation of a method for the determination of catechin and epicatechin in red wines by HPLC-FL and reported analytical curves between 1 and 30 μg mL^{-1}. Careri et al. [31] demonstrated linearity over the 0.39 to 12.5 μg mL^{-1} and 0.45 to 57.6 μg mL^{-1} range for trans-resveratrol and quercetin, respectively, in a validation for the matrices red wine, grape, and winemaking byproducts by HPLC-DAD. However, these studies only considered the coefficient of determination for the linearity assessment. Despite the current widespread use of this coefficient as an indication of quality of fit, it is misleading and inappropriate as a test for linearity and should not be used [23].

3.2. Matrix effects

All the regression assumptions were confirmed for the solvent and matrix-matched calibration curves. No significant matrix effects were detected when the slopes of the solvent and matrix-matched curves were compared, for the 18 phenolic compounds, in the studied ranges ($p > 0.05$). For 7 phenolic compounds (cyanidin-3-glucoside-chloride, malvidin-3,5-di-O-glucoside-chloride, pelargonidin-3-O-glucoside-chloride, isorhamnetin-3-O-glucoside, resveratrol, chlorogenic acid and gallic acid), absence of matrix effects ($p > 0.05$) only was observed for a reduced concentration range, as presented in Table 4. Therefore, solvent curves were used to estimate the phenolic concentration in grape juice samples, considering the appropriate concentration range for each analyte.

Munõz et al. [18] compared the response obtained when using different standards of anthocyanins with that obtained for malvidin-3-glucoside (usual calibration standard). The comparison of the graph slopes confirmed the different behavior of the studied anthocyanins. Despite of this comparison, matrix effects were not investigated. The study of this parameter is often neglected in validation processes, although, the evaluation is strongly recommended, mainly when dealing with complex matrixes. According to Thompson et al. [23] the calibration is enormously simplified if the calibration standards can be prepared as simple solutions of the analyte. Then, the effects of a possible general matrix mismatch must be assessed in validation if this strategy is adopted.

3.3. Trueness and precision

The individual recovery values obtained for the spiked samples are shown in Fig. 2.

The mean recovery values ranged from 98.27 to 102.01%, 86.18 to 106.50%, 83.97 to 100.93% and 86.86 to 97.10% for anthocyanins, flavonols, phenolic acids and tannins, respectively (Fig. 2). These results demonstrated no lack of trueness of the validated method.

RSD_r varied from 0.73 to 2.87% for unspiked samples and from 0.71 to 9.24% for spiked samples, while RSD_R were between 1.99 and 6.46% for unspiked samples and between 1.34 and 9.26% for spiked samples (Table 5). The estimated RSD_r and RSD_R were lower than the respective critical values for all studied analytes, indicating precision of the method.

Although Fracassetti et al. [29], Dias et al. [30], Muñoz et al. [18] and Careri et al. [31] have studied a more restricted scope of analytes and only investigated precision under repeatability conditions, the reported recoveries and RSD_r were similar to those found in this paper.

3.4. Limits of detection and quantification

The theoretical limits of detection and quantification ranged between 0.001 and 0.19 μg mL^{-1} and between 0.003 and 0.37 μg mL^{-1}, respectively (Table 5). These results suggested that the proposed method is appropriated for the detection and quantification of the 25 investigated phenolic compounds even in low concentration levels. In general, the limits of detection and quantification were lower than those published by Fracassetti et al. [29], Dias et al. [30], Muñoz et al. [18], Careri et al. [31] and Escarpa and González [13], that were based on signal/noise ratio of 3 and 10, respectively.

3.5. Characterization of grape juice

The concentrations of the 25 phenolic compounds investigated for the seven samples of grape juices produced in SFV are shown in Table 6. The anthocyanins were the main phenolic class detected in *V. labrusca*, hybrid, Tempranillo and Alicante Bouschet grape juices, corresponding to 44.35 to 70.33% of the total phenolic content quantified for the samples. These results were consistent with those described by Rodrigues et al. [32] and Stalmach et al. [33]. It is known that the anthocyanins are responsible for the coloration of the grape, have the ability to scavenge the excess radicals and may play a role in the prevention of diseases [34–36].

The anthocyanINS cyanidin-3,5-diglucoside-chloride and cyanidin-3-glucoside-chloride were quantified in high concentrations in BRS Violeta grape juice. However, they were absent or present in low concentration in other studied juices. In BRS Cora juice, the cyanidin-3,5-diglucoside-chloride represented the major anthocyanin. This profile was also found by Xu et al. [36]. However, the values reported by these authors were smaller than those detected for BRS Violeta juice.

Delphinidin-3-glucoside-chloride was the anthocyanin quantified in BRS Violeta and BRS Cora juices in higher concentrations and determined in lower concentrations in other red juices. Considering the Tiwari et al. [19] results, this anthocyanin also appeared in low levels in the *V. vinifera* L. red juice evaluated. Already the malvidin diglucoside was found in higher concentration in BRS Violeta juice, while a monoglucoside form appeared in the Isabel Precoce and *V. vinifera* L. red juices. These results agreed with the data reported by Tenore et al. [5], that identified malvidin-3-O-glucoside as the main anthocyanin in *V. vinifera* red juices.

Pelargonidin-3-O-glucoside chloride was quantified mainly in BRS Cora juice, a profile also described by Wang et al. [37] during the assessment of juices produced with grapes from the same species. Peonidin-3-O-glucoside chloride was the most abundant anthocyanin of Alicante Bouschet juice, being also quantified in the other juices, except Moscato

Table 3
Evaluation of the regression assumptions for the solvent calibration curves — linearity assessment.

Phenolic compounds	Equation	R^2[a]	n[b]	Normality		Independency		Homoscedasticity		Regression		Lack-of-fit	
				R[c]	p[d]	d[e]	p[d]	t_L[f]	p[d]	F[g]	p[d]	F[g]	p[d]
Anthocyanins													
Cyanidin-3,5-diglucoside-chloride	$y = 22844x + 10748$	0.9984	12	0.9803	$p > 0.10$	2.08	$p > 0.05$	2.17	0.06	6209.15	2.65×10^{-15}	2.13	0.19
Cyanidin-3-glucoside-chloride	$y = 45405x + 16107$	0.9984	12	0.9873	$p > 0.10$	2.23	$p > 0.05$	1.07	0.31	6137.03	2.81×10^{-15}	1.70	0.27
Delphinidin-3-glucoside-chloride	$y = 37747x + 17541$	0.9990	12	0.9852	$p > 0.10$	2.19	$p > 0.05$	1.35	0.21	10321.01	2.09×10^{-16}	1.32	0.36
Malvidin-3,5-di-O-glucoside-chloride	$y = 42295x + 16847$	0.9990	12	0.9817	$p > 0.10$	2.49	$p > 0.05$	0.79	0.45	10130.30	2.30×10^{-16}	1.60	0.29
Malvidin-3-glucoside-chloride	$y = 55142x + 9098$	0.9976	12	0.9903	$p > 0.10$	2.03	$p > 0.05$	0.70	0.50	4080.11	2.15×10^{-14}	1.34	0.36
Pelargonidin-3-O-glucoside-chloride	$y = 55444x + 5843.9$	0.9995	12	0.9388	$p > 0.05$	1.93	$p > 0.05$	1.05	0.32	20251.03	7.21×10^{-18}	4.07	0.06
Peonidin-3-O-glucoside-chloride	$y = 67001x + 2076.4$	0.9976	11	0.9563	$p > 0.10$	2.77	$p > 0.025$	0.11	0.91	3813.06	3.86×10^{-13}	1.09	0.45
Flavonols													
Isorhamnetin-3-O-glucoside	$y = 43690x + 2006.7$	0.9988	11	0.9686	$p > 0.10$	3.03	$p < 0.01$	1.10	0.30	7678.24	1.66×10^{-14}	0.57	0.69
Kaempferol-3-O-glucoside	$y = 25426x + 1397.2$	0.9987	12	0.9812	$p > 0.10$	3.22	$p < 0.01$	0.99	0.35	7639.29	9.40×10^{-16}	0.37	0.82
Myricetin	$y = 75965x + 1050.5$	0.9992	11	0.9822	$p > 0.10$	2.72	$p > 0.025$	0.26	0.80	10954.58	3.37×10^{-15}	0.36	0.83
Quercetin	$y = 83734x + 2500.1$	0.9996	11	0.9656	$p > 0.10$	1.89	$p > 0.05$	0.98	0.35	21818.64	1.52×10^{-16}	1.51	0.33
Resveratrol	$y = 147196x + 12633$	0.9991	12	0.9816	$p > 0.10$	2.60	$p > 0.05$	0.83	0.43	10594.86	1.84×10^{-16}	1.56	0.30
Rutin	$y = 40716x + 1009.7$	0.9998	12	0.9753	$p > 0.10$	2.32	$p > 0.05$	0.69	0.51	8456.23	5.66×10^{-16}	0.73	0.60
Phenolic acids													
Caffeic acid	$y = 121831x + 26031$	0.9988	11	0.9838	$p > 0.10$	3.05	$p < 0.01$	0.98	0.35	7457.53	1.90×10^{-14}	0.72	0.62
Chlorogenic acid	$y = 65252x + 27046$	0.9986	11	0.9669	$p > 0.10$	2.07	$p > 0.05$	0.31	0.76	6365.18	3.87×10^{-14}	2.12	0.21
Cinnamic acid	$y = 113729x + 15360$	0.9993	11	0.9443	$p > 0.10$	3.26	$p < 0.01$	2.18	0.06	12052.62	2.19×10^{-15}	0.77	0.59
Gallic acid	$y = 173091x + 10779$	0.9987	11	0.9814	$p > 0.10$	2.57	$p > 0.05$	1.09	0.30	6901.70	2.69×10^{-14}	1.41	0.35
p-coumaric acid	$y = 137748x + 25549$	0.9999	9	0.9802	$p > 0.10$	1.50	$p > 0.05$	0.20	0.85	52716.26	7.85×10^{-15}	1.97	0.30
Tannins													
(−)-epicatechin	$y = 1 \times 10^7x + 5 \times 10^6$	0.9838	12	0.9533	$p > 0.10$	1.69	$p > 0.05$	1.03	0.33	605.69	2.80×10^{-10}	2.80	0.13
(−)-epicatechin gallate	$y = 122591x - 2556.5$	0.9962	11	0.9806	$p > 0.10$	2.69	$p > 0.025$	0.02	0.98	2336.77	3.38×10^{-12}	0.80	0.58
(−)-epigallocatechin gallate	$y = 132703x - 5981.3$	0.9993	10	0.9305	$p > 0.05$	2.80	$p > 0.05$	2.15	0.06	11180.83	7.15×10^{-14}	0.44	0.78
(+)-catechin	$y = 1 \times 10^7x + 6 \times 10^6$	0.9887	12	0.9223	$p > 0.01$	1.85	$p > 0.05$	0.77	0.46	873.26	4.60×10^{-11}	1.58	0.29
Procyanidin A2	$y = 8 \times 10^6x + 2 \times 10^6$	0.9901	12	0.9723	$p > 0.10$	2.27	$p > 0.05$	0.85	0.42	995.57	2.40×10^{-11}	1.52	0.31
Procyanidin B1	$y = 80529x + 8156.9$	0.9997	9	0.9716	$p > 0.10$	2.99	$p < 0.01$	0.35	0.74	20661.29	2.08×10^{-13}	0.17	0.94
Procyanidin B2	$y = 7 \times 10^6x + 4 \times 10^6$	0.9839	12	0.9484	$p > 0.10$	2.31	$p > 0.05$	0.83	0.43	610.38	2.70×10^{-10}	1.54	0.30

[a] Coefficient of determination.
[b] Number of observations.
[c] Ryan–Joiner correlation coefficient.
[d] Significance.
[e] Durbin–Watson statistic.
[f] Levene *t*-statistic.
[g] Variance ratio.

Table 4
Slope comparison of solvent and matrix-matched calibration curves.

Phenolic compounds[a]	Slope comparisons of solvent and matrix-matched curve		
	t_c[b]	t[c]	p[d]
Anthocyanins			
Cyanidin-3,5-diglucoside-chloride	2.31	0.01	0.99
Cyanidin-3-glucoside-chloride	2.13	1.87	0.08
Delphinidin-3-glucoside-chloride	2.10	1.16	0.26
Malvidin-3,5-di-O-glucoside-chloride	2.14	0.20	0.84
Malvidin-3-glucoside-chloride	2.26	0.66	0.53
Pelargonidin-3-O-glucoside-chloride	2.13	1.33	0.20
Peonidin-3-O-glucoside-chloride	2.26	0.12	0.90
Flavonols			
Isorhamnetin-3-O-glucoside	2.57	2.11	0.09
Kaempferol-3-O-glucoside	2.10	0.12	0.91
Myricetin	2.23	0.27	0.79
Quercetin	2.09	1.54	0.14
Resveratrol	2.31	0.31	0.76
Rutin	2.20	0.24	0.81
Phenolic acids			
Caffeic acid	2.18	0.87	0.40
Chlorogenic acid	2.45	1.79	0.12
Cinnamic acid	2.18	1.30	0.22
Gallic acid	2.31	1.70	0.13
p-coumaric acid	2.16	0.46	0.65
Tannins			
(−)-epicatechin	2.09	1.65	0.11
(−)-epicatechin gallate	2.11	0.07	0.94
(−)-epigallocatechin gallate	2.20	1.06	0.31
(+)-catechin	2.10	0.30	0.77
Procyanidin A2	2.09	0.94	0.36
Procyanidin B1	2.09	1.41	0.17
Procyanidin B2	2.09	0.97	0.34

[a] Phenolic compounds for which the concentration ranges were modified:
[b] t critical value.
[c] t-statistic for the contrasts of the matrix-matched curves with the solvent curve.
[d] Significance.

Canelli. In other samples, this anthocyanin was present in lower concentrations. In the Moscato Canelli juice anthocyanins were not identified, which was expected due to the absence of red coloration on this grape. These data demonstrated that the profile of anthocyanins is largely influenced by the cultivar of grape used in the production of the juice.

The flavonols were the phenolic class present in lower concentration in the studied juices, which total content ranged from 0.56 to 11.71%. Mulero et al. [8] affirmed that the flavonols are present in much smaller quantities in red grape that anthocyanins. However, this group of flavonoids has been one of the most studied groups because of their biological effects and antioxidant potential [38].

Syrah juice was the sample with higher flavonol contents, especially isorhamnetin-3-O-glucoside, which also predominated in Tempranillo juice. The highest levels of kaempferol-3-O-glucoside were quantified in Isabel Precoce, Syrah and Alicante Bouschet juices. On the other hand, myricetin showed higher content in the BRS Violeta juice.

Quercetin was not detected or was quantified in low concentration in most of the analyzed samples. Resveratrol was identified, also in low concentration, in a few samples, confirming what was found and published by Rodrigues et al. [32] for Brazilian Bordeaux juices. As described by other authors [9,38], the major flavonols commonly found in vegetal tissues are glycosylated, confirming the results obtained in this study, once resveratrol and quercetin were investigated in aglycone forms. Rutin was a flavonol found in higher concentrations in hybrid juice. Values obtained in this study for flavonols were similar to those previously reported for Concord grape juice by Stalmach et al. [33].

The identified phenolic acids represented from 1.07 to 33.82% of the total phenolics in the studied samples, highlighted in Moscato Canelli juice. The phenolic acids were one of the major non-flavonoid phenolic compounds quantified in grapes, that had shown important biological effects [39]. The caffeic acid was the predominant phenolic acid in Isabel Precoce and Violeta juices. Chlorogenic acid showed homogeneous distribution in red grape juices and was determinated in low level in white grape juice. Cinnamic acid was detected in BRS Violeta and Tempranillo juices, which levels were in accordance with the values described by Ganic et al. [40].

In the *V. vinifera* L. red grape juices, the gallic acid was the most abundant phenolic acid. This phenolic is the main phenolic in grape seeds and has antioxidant and antifungal activities [41]. In the Moscato Canneli juice, the gallic acid accounted for more than 94% of the phenolic acids present. The values found in this study were consistent with those reported by Rodrigues et al. [32]. The *p*-coumaric acid content was more representative in BRS Violeta, Alicante Bouschet and Isabel Precoce juices. In these samples, the *p*-coumaric acid was identified in levels three times higher than those described for grape juices [40].

The tannins represented from 11.84 to 58.68% of phenolic total content. The compounds of this class are mainly responsible for astringency and bitterness of grapes and its consumption is related with positive effects of the prevention of chronic diseases [14,15,42]. In Syrah and Moscato Canelli juices, this phenolic class was predominant.

The (−)-epicatechin levels found in the BRS Violeta, Syrah and Alicante Bouschet were higher than those observed in other samples. These values were close to (−)-epicatechin levels described for wine produced with *V. vinifera* grape in the São Francisco Valley [30]. Low or trace amounts of the (−)-epicatechin gallate and (−)-epigallocatechin

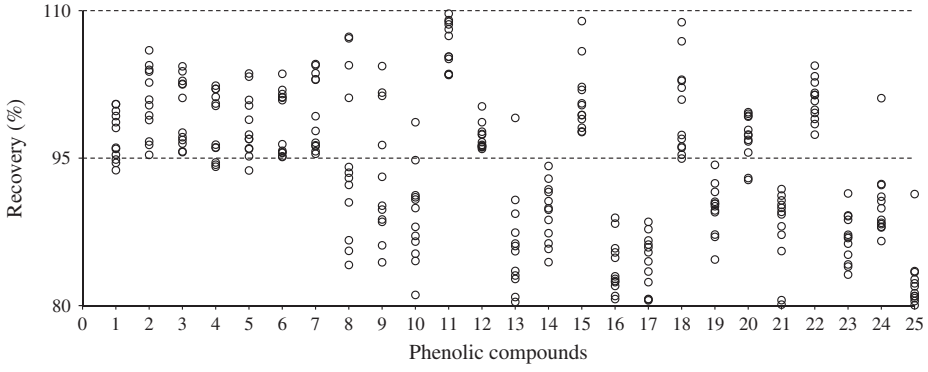

Fig. 2. Individual recovery percentage obtained for the twelve replicates of spiked samples, analyzed under within-reproducibility conditions. Anthocyanins — 1: cyanidin-3,5-diglucoside-chloride; 2: cyanidin-3-glucoside-chloride; 3: delphinidin-3-glucoside-chloride; 4: malvidin-3,5-di-O-glucoside-chloride; 5: malvidin-3-glucoside-chloride; 6: pelargonidin-3-O-glucoside-chloride; 7: peonidin-3-O-glucoside-chloride. Flavonols — 8: isorhamnetin-3-O-glucoside; 9: kaempferol-3-O-glucoside; 10: myricetin; 11: quercetin; 12: resveratrol; 13: rutin. Phenolic acids — 14: caffeic acid; 15: chlorogenic acid; 16: cinnamic acid; 17: gallic acid; 18: p-coumaric acid. Tannins — 19: (−)-epicatechin; 20: (−)-epicatechin gallate; 21: (−)-epigallocatechin gallate; 22: (+)-catechin; 23: procyanidin A2; 24: procyanidin B1; 25: procyanidin B2.

Table 5
Relative standard deviation under repeatability and within-reproducibility conditions and limits of detection and quantification obtained for the different phenolic compounds.

Phenolic compounds	Unspiked samples					Spiked samples					Detection limit (μg mL^{-1})	Quantification limit (μg mL^{-1})
	C_T[a] (μg mL^{-1}of grape juice)	RSD_r[b] (%)		RSD_R[c] (%)		C_T[a] (μg mL^{-1}of grape juice)	RSD_r[b] (%)		RSD_R[c] (%)			
		Critical value	Calculated value	Critical value	Calculated value		Critical value	Calculated value	Critical value	Calculated value		
Anthocyanins												
Cyanidin-3,5-diglucoside-chloride	2.02	3.77	0.73	5.65	4.05	12.02	8.14	0.71	12.21	2.77	0.04	0.14
Cyanidin-3-glucoside-chloride	14.54	2.80	2.11	4.20	2.76	18.54	7.63	3.89	11.44	3.89	0.11	0.37
Delphinidin-3-glucoside-chloride	10.88	2.92	2.79	4.38	3.14	14.88	7.88	2.84	11.83	3.55	0.09	0.28
Malvidin-3,5-di-O-glucoside-chloride	20.02	2.67	1.64	4.00	1.99	24.02	7.62	3.22	11.44	3.44	0.06	0.21
Malvidin-3-glucoside-chloride	5.02	3.28	2.43	4.92	3.87	7.03	3.28	2.43	4.92	3.87	0.03	0.12
Pelargonidin-3-O-glucoside chloride	6.92	3.13	2.87	4.69	3.02	7.92	8.66	3.27	12.98	3.33	0.04	0.12
Peonidin-3-O-glucoside chloride	1.94	3.79	1.67	5.68	2.52	2.44	10.31	2.84	15.47	3.94	0.01	0.08
Flavonols												
Isorhamnetin-3-O-glucoside	1.90	3.80	2.14	5.70	2.14	2.40	10.15	4.83	15.22	9.26	0.01	0.04
Kaempferol-3-O-glucoside	2.50	3.64	2.70	5.47	2.70	3.00	10.03	9.24	15.05	9.24	0.06	0.21
Myricetin	0.24	5.18	2.31	7.78	6.46	1.49	11.15	5.38	16.72	5.38	0.01	0.03
Quercetin	ND	–	–	–	–	1.25	11.45	1.97	17.17	2.28	0.002	0.01
Resveratrol	ND	–	–	–	–	2.50	10.31	1.29	15.47	1.34	0.01	0.05
Rutin	1.44	3.96	1.59	5.94	3.11	1.94	10.71	3.55	16.07	6.37	0.04	0.13
Phenolic acids												
Caffeic acid	9.46	2.98	1.42	4.48	2.55	11.46	8.20	2.16	12.30	3.58	0.08	0.28
Chlorogenic acid	3.60	3.45	0.85	5.80	2.08	8.60	8.56	3.56	12.84	3.56	0.02	0.07
Cinnamic acid	ND	–	–	–	–	5.00	9.29	2.75	13.94	3.33	0.11	0.37
Gallic acid	ND	–	–	–	–	2.50	10.31	3.14	15.47	3.40	0.07	0.24
p-coumaric acid	1.82	3.83	2.81	5.74	3.13	6.82	8.87	4.49	13.31	4.72	0.05	0.16
Tannins												
(−)-epicatechin	ND	–	–	–	–	5.00	3.29	1.48	4.93	3.08	0.01	0.04
(−)-epicatechin gallate	ND	–	–	–	–	2.50	10.31	2.58	15.47	2.58	0.07	0.10
(−)-epigallocatechin gallate	3.92	3.41	1.70	5.11	3.51	4.92	9.31	4.85	13.97	4.85	0.19	0.31
(+)-catechin	2.48	3.64	2.27	5.45	4.10	7.54	8.73	1.81	13.10	2.10	0.01	0.03
Procyanidin A2	ND	–	–	–	–	2.50	10.31	2.90	15.47	2.90	0.003	0.01
Procyanidin B1	2.14	3.74	1.65	5.60	3.32	2.64	10.23	3.81	15.34	4.30	0.03	0.11
Procyanidin B2	5.18	3.27	2.72	4.90	3.61	7.18	8.79	3.47	13.19	3.69	0.001	0.003

[a] C_T: theoretical concentration (native concentration for unspiked samples and native plus spiked concentration for spiked samples).
[b] Relative standard deviation under repeatability conditions.
[c] Relative standard deviation under within-reproducibility.

Table 6
Phenolic compound < LDs profile of *Vitis labrusca*, hybrid a < LD *Vitis vinifera* grape juices from São Francisco Valley, Brazil.

Phenolic compound < LDs	*Vitis labrusca* grape juices		Hybrid grape juice	*Vitis vinifera* grape juices			
	Isabel Precoce (mg L^{-1})	BRS Cora (mg L^{-1})	BRS Violeta (mg L^{-1})	Tempranillo (mg L^{-1})	Syrah (mg L^{-1})	Alicante Bouschet (mg L^{-1})	Moscato Canelli (mg L^{-1})
Anthocyanins							
Cyanidin-3,5-diglucoside-chloride	1.80 ± 0.15 ^{c}	22.51 ± 3.32 ^{b}	192.06 ± 12.20 ^{a}	<LD	<LD	0.63 ± 0.08 ^{c}	<LD
Cyanidin-3-glucoside-chloride	8.80 ± 0.89 ^{b}	<LD	116.67 ± 7.84 ^{a}	<LD	<LD	<LQ	<LD
Delphinidin-3-glucoside-chloride	7.04 ± 1.22 ^{c}	32.19 ± 4.21 ^{b}	270.45 ± 13.83 ^{a}	9.82 ± 3.11 ^{c}	3.15 ± 0.72 ^{c}	2.39 ± 0.51 ^{c}	<LD
Malvidin-3,5-di-O-glucoside-chloride	15.68 ± 1.16 ^{b}	4.95 ± 0.53 ^{c}	75.42 ± 8.92 ^{a}	1.69 ± 0.59 ^{c}	0.40 ± 0.08 ^{c}	5.08 ± 0.85 ^{c}	<LD
Malvidin-3-glucoside-chloride	24.01 ± 2.51 ^{a}	0.52 ± 0.08 ^{d}	5.51 ± 0.64 ^{c}	20.61 ± 6.17 ^{ab}	18.84 ± 5.07 ^{b}	24.30 ± 1.94 ^{a}	<LD
Pelargonidin-3-O-glucoside chloride	6.82 ± 0.61 ^{bc}	14.47 ± 1.56 ^{a}	7.85 ± 1.07 ^{b}	6.06 ± 1.82 ^{c}	3.16 ± 0.73 ^{d}	3.26 ± 0.52 ^{d}	<LD
Peonidin-3-O-glucoside chloride	9.33 ± 0.76 ^{b}	0.15 ± 0.06 ^{d}	1.44 ± 0.13 ^{cd}	3.06 ± 0.94 ^{c}	2.75 ± 0.55 ^{c}	34.18 ± 4.34 ^{a}	<LD
% of total composition	70.33	70.09	52.76	45.82	23.78	44.35	<LD
Flavonols							
Isorhamnetin-3-O-glucoside	1.90 ± 0.04 ^{c}	0.67 ± 0.17 ^{d}	0.39 ± 0.00 ^{d}	2.47 ± 0.35 ^{c}	10.56 ± 1.75^{a}	5.14 ± 0.47 ^{b}	<LD
Kaempferol-3-O-glucoside	2.48 ± 0.04 ^{a}	0.93 ± 0.25 ^{c}	<LD	<LD	2.38 ± 0.48 ^{a}	1.64 ± 0.23 ^{b}	0.40 ± 0.02 ^{d}
Myricetin	0.24 ± 0.01 ^{c}	0.20 ± 0.03 ^{c}	1.25 ± 0.17 ^{a}	0.47 ± 0.02 ^{b}	0.46 ± 0.06 ^{b}	0.28 ± 0.03 ^{c}	<LD
Quercetin	<LD	0.48 ± 0.10 ^{a}	<LD	0.04 ± 0.01 ^{b}	<LD	<LD	<LD
Resveratrol	0.05 ± 0.01 ^{c}	<LD	0.40 ± 0.11 ^{b}	<LD	<LD	0.67 ± 0.06 ^{a}	<LD
Rutin	1.49 ± 0.04 ^{b}	0.98 ± 0.15 ^{c}	5.09 ± 0.84 ^{a}	0.74 ± 0.13 ^{c}	0.54 ± 0.11 ^{cd}	0.55 ± 0.10 ^{cd}	0.17 ± 0.06 ^{d}
% of total composition	5.90	3.06	0.56	4.13	11.71	5.26	7.50
Phenolic acids							
Caffeic acid	7.30 ± 0.38 ^{a}	2.62 ± 0,16 ^{d}	4.54 ± 1.14 ^{b}	2.45 ± 0.19 ^{d}	3.72 ± 0.42 ^{c}	3.57 ± 0.46 ^{c}	<LD
Chlorogenic acid	3.90 ± 0.15 ^{a}	2.36 ± 0.23 ^{b}	2.24 ± 0.47 ^{b}	3.60 ± 0.43 ^{a}	2.45 ± 0.44 ^{b}	2.54 ± 0.45 ^{b}	0.14 ± 0.09 ^{c}
Cinnamic acid	<LD	<LQ	1.66 ± 0.48 ^{a}	1.69 ± 0.31 ^{a}	<LQ	<LD	<LD
Gallic acid	<LD	1.92 ± 0.60 ^{d}	3.11 ± 0.79 ^{c}	4.25 ± 0.30 ^{b}	5.42 ± 0.49 ^{a}	4.47 ± 0.40 ^{b}	2.43 ± 0.14 ^{cd}
p-coumaric acid	1.27 ± 0.18 ^{b}	0.28 ± 0.05 ^{c}	2.08 ± 0.91 ^{a}	<LD	0.07 ± 0.04 ^{c}	1.94 ± 0.31 ^{a}	<LD
% of total composition	11.93	6.73	1.07	13.32	9.80	7.95	33.82
Tannins							
(−)-epicatechin	<LD	1.36 ± 0.12 ^{c}	36.46 ± 7.75 ^{a}	3.56 ± 0.47 ^{c}	22.17 ± 9.41 ^{b}	17.74 ± 2.54 ^{b}	1.30 ± 0.47 ^{c}
(−)-epicatechin gallate	<LD	<LD	0.14 ± 0.00 ^{c}	4.88 ± 0.29 ^{a}	4.80 ± 0.85 ^{a}	1.12 ± 0.08 ^{b}	<LD
(−)-epigallocatechin gallate	0.63 ± 0,10 ^{c}	6.20 ± 1.63 ^{a}	1.77 ± 0.27 ^{b}	2.39 ± 0.22^{b}	<LD	0.68 ± 0.10 ^{c}	0.60 ± 0.04 ^{c}
(+)-catechin	3.38 ± 0.45 ^{c}	3.31 ± 0.29 ^{c}	249.03 ± 19.86 ^{a}	8.25 ± 1.20 ^{c}	24.83 ± 11.95 ^{b}	16.40 ± 2.22 ^{bc}	2.20 ± 0.98 ^{c}
Procyanidin A2	<LD	<LD	1.43 ± 0.38 ^{a}	<LD	0.30 ± 0.03 ^{c}	0.92 ± 0.18 ^{b}	0.36 ± 0.03 ^{c}
Procyanidin B1	4.82 ± 0.47 ^{d}	5.90 ± 0.83 ^{cd}	18.36 ± 4.82 ^{a}	9.97 ± 1.40 ^{b}	8.72 ± 0.31 ^{bc}	17.63 ± 0.52 ^{a}	<LD
Procyanidin B2	3.54 ± 0.62 ^{b}	4.70 ± 0.56 ^{b}	271.38 ± 17.44 ^{a}	4.03 ± 0.44 ^{b}	4.28 ± 1.18 ^{b}	12.35 ± 0.76 ^{b}	<LD
% of total composition	11.84	20.12	45.60	36.75	54.71	42.44	58.68
Total phenolics (mg L^{-1})	104.48	106.70	1268.73	90.03	119.00	157.48	7.60

LD: detection limit. LQ: quantification limit. NQ: not quantified. Values were expressed as mean of nine replicates ± sta < LDard deviation. Values within each row followed by the different superscript letter have significant difference ($p < 0.05$).

gallate were detected in the studied samples, with concentrations ranging from 0.0 to 6.20 mg L^{-1}. Ganic et al. [40] also found less expressive levels of (−)-epicatechin gallate in Malvasia istriana juice.

The BRS Violeta juice exhibited significant levels of (+)-catechin. This phenolic was the predominant tannin in Moscato Canelli juice. In contrast to the literature, the (+)-catechin level in the BRS Violeta juice was between ten and twenty times larger than the levels reported for red grape juice [32,40]. The three researched procyanidin (A2, B1 and B2) appeared in higher concentration in BRS Violeta juice and were not detected or quantified in low levels in white grape juice. In the other juice samples, the procyanidin B1 and B2 showed higher values than that obtained for procyanidin A2.

Mulero et al. [8] affirmed that several factors may influence the concentration of phenolic compounds, including the grape cultivar. This fact was confirmed in this study, once the concentration and phenolic profile of samples varied according to the grape variety.

4. Conclusions

The validated method was reliable for the determination of phenolic profile in grape juices, including authenticity and quality control purposes. The main advantages of the developed method are the simultaneous identification of 25 phenolic compounds, belonging to four different classes, in a single chromatographic run, the low costs of the solvents and materials and the simplicity of the sample preparation step. The SFV grape juice phenolic composition varied in function of the grape cultivar. Anthocyanins and tannins were the most abundant phenolic classes identified in red grape juices, while flavonols were found in lower concentrations. Considering the white grape juice, tannins and phenolic acids were detected in higher concentrations. These findings suggest that the preparation of beverages based on the grape blends may be an alternative to improve their functional potential.

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