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Determination of Quercetin, Gallic Acid, Resveratrol, Catechin and Malvidin in Brazilian Wines Elaborated in the Vale do São Francisco Using Liquid–Liquid Extraction Assisted by Ultrasound and GC-MS

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Abstract In this work, a fast and simple methodology has been applied for the determination of gallic acid, resveratrol, catechin and malvidin in Brazilian wines by gas chromatography–mass spectrometry. The procedure included a stage of ultrasound-assisted liquid–liquid extraction and subsequent derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and GC-MS analysis. The limit of detection varied from 0.41 to 1.18 mgL^{-1} in all the analytes. The relative standard deviations calculated for 8.0 and 20 mgL^{-1} were 1.90 and 0.82 % for gallic acid, 3.08 and 1.22 % for catechin, 1.30 and 0.44 % for malvidin, 1.50 and 0.53 % for resveratrol, and 1.41 and 0.61 % for quercetin. The developed methodology was applied for the analysis of red wine samples collected in the São Francisco region, Bahia state, Brazil. Quercetin concentration varied from 2.4 to 3.0 mg L^{-1}, gallic acid 21.4–56.3 mgL^{-1}, resveratrol 1.5–5.9 mg L^{-1}, malvidin 15.3–32.2 mgL^{-1}, and catechin 11.71–18.2 mgL^{-1}. The obtained concentrations are in agreement with those reported in the literature.

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Introduction

Phenolic compounds constitute one of the most important quality parameters of wine since these compounds have a great impact on the sensorial characteristics, especially color and flavor. Several studies have firmly established (Saint-Cricq et al. 1999; Tsao et al. 2005) that the moderate consumption of wine contributes to the improvement of human health; phenolic substances have demonstrated antiseptic and antiviral activities and could therefore prevent the vascular diseases by protecting blood vessels (Stoclet et al. 2004). The recent interest in these phenolic constituents of red wine, including gallic acid, resveratrol, quercetin, and rutin has been stimulated by their multiple biological effects, such as antioxidant activity (Feliciano et al. 2009), anti-inflammatory action, platelet aggregation inhibition, and antimicrobial activities (Goldberg et al. 1999). Polyphenols also play an important role in enology, as they importantly contribute to the color and sensory properties of wine. The synthesis and accumulation of phenolic compounds in grapes is primarily dependent upon varietal factors whose expression is influenced by a combination of climatic and viticultural factors.

Wines have been analyzed by liquid chromatography (Pereira et al. 2010; Nixdorf and Hermosín-Gutiérrez 2010; Aznar et al. 2011; Gallego et al. 2011; Kelebek et al. 2011) with diode array/fluorescence (Vitrac et al. 2002; Dias et al. 2010) and electrochemical detection (Bocchi et al. 1996; Kolouchová-Honzlíková et al. 2004). Atmospheric pressure ionization–liquid chromatography (electrospray or

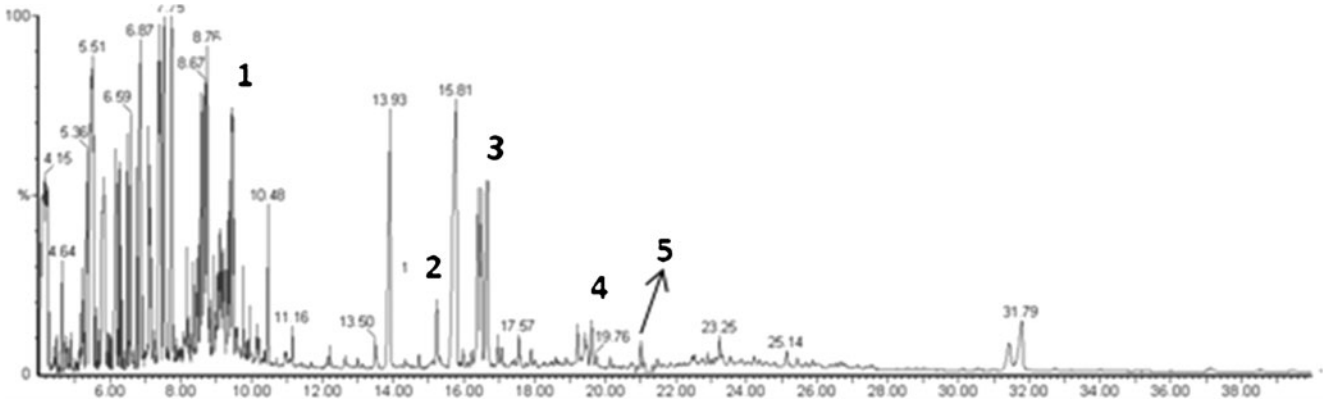

Fig. 1 Total ion chromatograms: *1* Gallic acid, *2* Resveratrol, *3* Catechin, *4* Quercetin, *5* Malvidin

chemical ionization) has also been used (Flamini 2003; Spácil et al. 2008) to identify the chemical structures of wine phenolic compounds. This technique frequently enables the determination of low concentration levels on one analyzed analyte, in the presence of many other interfering and coeluting components, which is valuable in combination with effective sample preparation techniques mainly in the online mode. It is generally known that the most difficult and time-consuming steps of the complete HPLC assay are the clean-up and preconcentration procedures of analytes from the biological matrix. The several clean-up/extraction/derivatization steps often involve multiple evaporations to dryness of the extract, employ large volumes of hazardous organic solvents, and are time consuming and/or expensive (Pascual-Martí et al. 2001).

Traditional separation techniques, including solvent extraction of the sample (e.g., in a Soxhlet extractor) followed by liquid–liquid extraction (Bru et al. 1996; Diaz et al. 2007), or column chromatography on different sorbents, such as chromatography in open columns using polyamide (Da Silva et al. 1990), are well-known procedures applied to the isolation and purification of plant phenolics. Subsequently, the extracts are analyzed by gas chromatography with flame ionization detection. This work addresses the use of ultrasound-assisted extraction using low-volume organic solvents from small sample amounts. The factors that influence both extraction/derivatization and chromatographic efficiencies have been critically assessed.

Table 1 Selective Ion monitoring of a target and qualifier ions for each phenolic compounds

Compounds	Base peak (BP) (*m*/*z*)	Qualifier ions (QI) (*m*/*z*)
Gallic acid	281	458;443
Resveratrol	444	428; 147
Catechin	650	368; 355; 267
Quercetin	647	645; 556; 392
Malvidin	619	589;295

Experimental

Reagents and Solvents

All chemicals were of the highest analytical grade available. Standards of gallic acid, resveratrol, catechin, malvidin and quercetin, were obtained from Sigma (St. Louis, MO, USA). The derivatizating reagent BSTFA was acquired from SUPELCO (Bellefonte, USA).

Sample Preparation

Seven samples of different types of red wine were purchased from commercial markets in Salvador (Bahia, Brazil). These samples were chosen as representatives of wines produced in the region of Vale do São Francisco-Brazil. All wines were stored in the dark, at 4 °C, until analysis.

Extraction and Derivatization Procedures

The following procedure for extraction of phenolic compounds in wine has been used: 4 mg of sodium chloride and 4 mg of sodium metabisulfite were added to 200 μL of wine. The samples were then subjected to three successive liquid

Table 2 Analytical features of the GC-MS method developed

Analyte	TR[a]	LOD[b]	LOQ[c]	Calibration data	R^2
Gallic acid	9.49	1.18	3.90	$S=1.25x10^7C+2.03\times10^7$	0.999
Resveratrol	15.1	0.41	1.36	$S=4.86x10^6C+1.28\times10^5$	0.997
Catechin	16.65	1.30	4.30	$S=8.49x10^7C-1.00\times10^8$	0.998
Quercetin	19.68	0.61	2.00	$S=7.60x10^7C-9.6\times10^6$	0.991
Malvidin	21.21	0.75	2.50	$S=1.22x10^5C-5.1\times10^5$	0.998

[a] Retention time (min)

[b] Detection limit (mgL^{-1})

[c] Quantification limit (mgL^{-1})

Table 3 Spike test for phenolic compounds concentrations (mgL^{-1}) in Brazilian wines obtained by the GC-MS method developed in region vale do São Francisco

	Content added (mgL^{-1})	Content found (mgL^{-1})	Rec. (%)
Gallic acid	0.0	6.20	98.0
	2.0	8.16	
Resveratrol	0.0	12.4	94.0
	5.0	17.10	
Catechin	0.0	1.65	107
	2.0	3.79	
Quercetin	0.0	2.63	93.5
	2.0	4.50	
Malvidin	0.0	11.7	104
	5.0	16.90	

extraction steps with 600 μL of acidified (with 37 % hydrochloric acid) ethyl acetate mediated by ultrasonication (200 W) for 7 min. After that, it was evaporated to dryness under a gentle stream of nitrogen. The solid residue was spiked with 30 μL of pyridine (Flucka, Steinheim, Germany), 70 μL of BSTFA, and 1 % trimethylsilyl (TMS). After 75 min at 70º C, an aliquot of 1 μL of derived extract was injected and analyzed by GC/MS. Each analysis was carried out in triplicate. Standard solutions were subjected to the same extraction/derivatization procedure as the samples.

Gas Chromatographic Analysis of Phenolic Compounds

The GC-MS analysis was carried out with a PerkinElmer apparatus, Model Clarus 500, and the used temperature program were the following: initial temperature of 80 °C, for 1 min, then from 80 to 250 °C at a rate of 20 $°C min^{-1}$, and held for 1 min; next, it was augmented at 6 $°C min^{-1}$ to 300 °C, held for 2 min, and finally increased at 20 $°C min^{-1}$ to 320 °C, and held for 24 min.

Results and Discussion

A 1-μL sample of each derivatized extract was injected to the GC-MS with the instrument on full-scan mode, from 100 to 700 amu. This allowed the establishment of the retention time (Fig. 1) and the characteristic TMS derivative mass spectrum of each phenolic compound (Table 1).

A six-point calibration curve was obtained from the standard solutions of phenolic compounds, which contained 15 % (*v*/*v*) of ethanol and were subjected to the same extraction–derivation procedure described for the samples. Quantification was performed by relating the peak areas of the identified compounds on each sample and concentrations were calculated from the calibration plot.

Validation Studies

The analytical curves were performed using standard solutions at different concentration ranges for each analyte. The corresponding regression equation and other characteristic parameters for the determination of phenolic compounds are shown in Table 2. The analytical curves exhibit excellent linear behavior over the concentration range under study.

Limit of detection and limit of quantification (LOQ) were established by analyzing the calibration curves (Numanoğlu et al. 2008).

The detection limits for all the analytes were found within the range 0.41–1.18 mgL^{-1}. The relative standard deviations calculated for 8.0 and 20 mgL^{-1} were found to be 1.90 and 0.82 % for gallic acid, 3.08 and 1.22 % for catechin, 1.30 and 0.44 % for malvidin, 1.50 and 0.53 % for resveratrol, and 1.41 and 0.61 % for quercetin. Accuracy was evaluated through addition/recovery tests. Absolute recoveries were evaluated by comparison of the concentrations found in four wine samples spiked with known amounts of each polyphenol. The results are given in Table 3. An aliquot of a standard mixture solution was added to different volumetric flasks so as to obtain a final concentration of 2.0, and 5.0 $mg.L^{-1}$. Finally, the volume was completed with a

Table 4 Phenolic compounds concentrations (mgL^{-1}) in Brazilian region vale do São Francisco wines obtained by the GC-MS method developed

Sample	Quercetin (mgL^{-1})	Gallic Acid (mgL^{-1})	Resveratrol (mgL^{-1})	Malvidin (mgL^{-1})	Catechin (mgL^{-1})
Shyraz	<LOQ	21.4±1.7	<LOQ	<LOQ	<LOQ
Cabernet Sauvignon	2.4±1.1	27.1±4.0	1.5±0.4	<LOQ	<LOQ
Cabernet Sauvignon/Shyraz	2.7±0.9	47.2±5.7	4.0±0.8	15.3±1.2	11.7±1.0
Cabernet Sauvignon/Shyraz	1.7±0.4	49.4±6.0	5.9±0.7	11.3±3.0	16.7±2.3
Shyraz	2.5±0.4	46.4±6.3	3.4±0.5	12.4±2.4	15.6±1.6
Shyraz	2.7±1.0	56.3±5.6	3.9±0.6	32.2±3.6	18.2±2.3
Cabernet Sauvignon/Shyraz	3.0±0.8	54.1±3.7	5.5±0.1	20.8±4.0	17.8±2.7

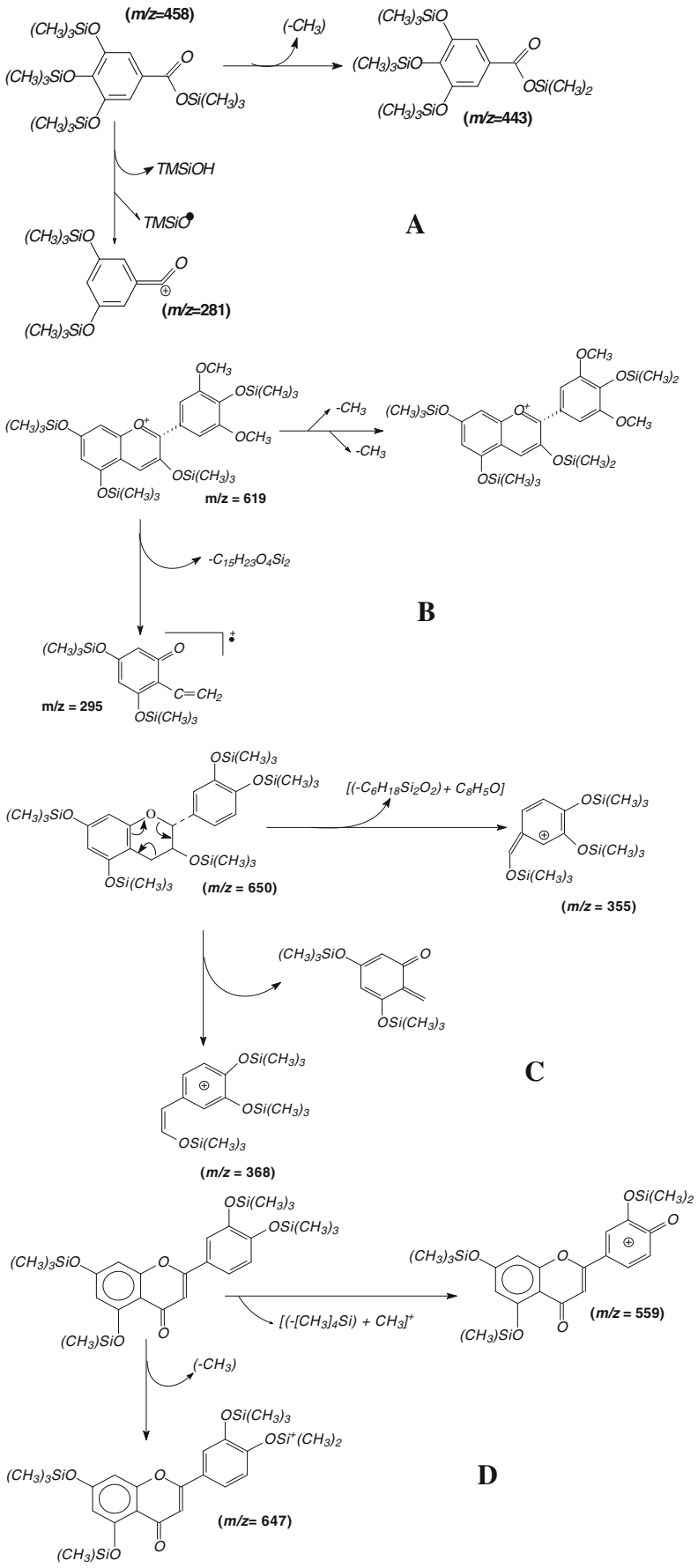

Fig. 2 *A* fragmentation of the derived TMS-gallic acid; *B* fragmentation of the derived TMS-malvidin; *C* fragmentation of the derived TMS-catechin; *D* fragmentation of the derived TMS-quercetin

wine sample and later analyzed in triplicate, according to the proposed method. For all the analyzed samples, the general results were of the same order as those reported in Table 4 for a wine sample.

Identification and Determination of Gallic Acid, Resveratrol, Catechin, Quercetin and Malvidin in Brazilian Wines

The mass fragments and retention times of derivatized patterns (Fig. 1) calculated on the base peak using single ion chromatograms have allowed for the identification of the following compounds: gallic acid, malvidin, catechin, resveratrol, and quercetin in wines produced in Vale do São Francisco.

By analyzing the mass fragmentation (Fig. 2(A)), we could note the presence of m/z=458 corresponding to the TMS-gallic acid derivative, whereas m/z=281 (base peak) corresponds to losses of TMSiOH and TMSiO mass fragments [M−$(CH_3)_3$SiOH+$(CH_3)_3$SiO]. The fragment m/z=443 corresponds to the loss of a methyl radical from $(CH_3)_3$SiO, $(CH_3)_3$SiO, [M−CH_3] (Fig. 2(A)). The mass fragmentation of TMS-malvidin presented on Fig. 2(B) indicates the presence of the fragment m/z=619, which is the base peak that corresponds to total mass of the compound TMS-malvidin. The mass fragmentation of TMS-catechin presented on Fig. 2(C) indicates the presence of the fragment m/z=368, which is the base peak that corresponds to a loss of the fragment m/z=282 [M−282], and the fragment m/z=355 originated from the retro Diels-Alder rearrangement. The f mass presented in the molecular ion m/z=444 corresponds to the derived TMS-resveratrol. The mass fragmentation of the derived TMS-quercetin (Fig. 2(D)) presents as base peak of m/z=647, which corresponds to the loss of a methyl group ([M−CH_3]) and the result of the fragmentation of the TMS-derived fragment m/z=559, which corresponds to the loss of (−$[CH_3]_4$Si+CH_3)$^+$).

Table 4 shows the concentrations of these compounds in seven Brazilian wines; quercetin concentration varied from 2.4 to 3.0 mgL^{-1}, gallic acid from 21.4 to 56.3 mgL^{-1}, resveratrol 1.5–5.9 mgL^{-1}, malvidin 15.3–32.2 mgL^{-1}, and catechin 11.71–18.2 mgL^{-1}.

The reported data on phenolic compounds for red wines found in the literature are usually within the following ranges: gallic acid (39.00–61.00 mgL^{-1}) (Castellari et al. 2002), quercetin (3.54–12.65 mgL^{-1}), catechin (17.72–41.87 mgL^{-1}), resveratrol (0.61–2.44 mgL^{-1}) (La Torre et al. 2006), and malvidin-3-*O*-glucoside (55.10 mg L^{-1}) (Alonso et al. 2007). The wide variation in phenolic compounds concentrations observed in this work can be partially explained by the analytical and natural variability of data in the levels of these compounds, as the phenolic composition of red wine is highly complex while its chemical composition is intimately correlated with the origin of the grapes, soil type, climate and production and conservation processes.

The reported data on phenolic compounds levels found for the wines produced in "Vale do São Francisco" are in accordance with the values described for wine samples of several countries.

Conclusion

In the present work, a simple, reliable, and sensitive method has been described to determine the phenolic compounds of red wines produced in the region of Vale do São Francisco. The values of gallic acid, resveratrol, catechin, malvidin, and quercetin found in the Brazilian red wines are in accordance with literature values for related compounds. The method was characterized by good precision, linearity, and accuracy. The procedure was applied to a wide range of red wines with the goal of providing a general knowledge of the composition of these antioxidants.

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