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Development and Validation of a Simple RP-HPLC-PDA Method for Determination of 18 Polyphenols in Grape Juice and Red Wine

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ABSTRACT

According to the trend of a healthy eating awareness trend, having a potential benefit on human health, some polyphenols like flavonoids, resveratrol, hydroxy-stilbenes, and phenolic acids are in the spotlight. Grapes, and one of the most widespread grape product wine; are among the best sources of these polyphenols. In this study, a highly specific, susceptible, and easy chromatographic method was brought out and validated to determine 18 polyphenols in grape and red wines. For this aim, an HPLC-PDA was used, and the separation was accomplished on an RP-ODS4 column. The method comprised of a mobile gradient phase consisting of A solution (acetic acid in water, pH 2.00) and a mixture of the solution A – acetonitrile (20:80, v/v), at a flow rate of 1.0 ml/min, and PDA detection was carried out at 260,280, 320, and 360 nm. According to the results, it can be said that the program indicated good linearity over the range of 1-40 mg L^{-1} of phenolics with r^2>0.99. The recovery of the 18 phenolics ranges from 83.17% to 119.88% at red wines and 88.20% to 117.83% at grape juices. The method is well precise, with the relative standard deviation (RSD) of the average concentration of the phenolic compounds are ranges from 1.22% to 2.02% at red wines and 1.01% to 2.56% at grape juices.

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Introduction

Phenolic compounds are congenitally components of grapes, and relevant products, especially wine, not only play an essential role in the organoleptic properties of both grapes and wine but also have health effects (Garrido and Borges, 2013; Pedroza et al., 2017). Phenolic compounds, mainly classified into flavonoids and non-flavonoids, are one of the most abundant groups of plant-derived secondary metabolites, generates the most critical quality parameters of wine because of their effects on organoleptic and nutritional characteristics (Burin et al., 2011; Gómez-Serranillos and González-Burgos, 2013; Briguglio et al., 2020). Grapes and their most popular product, wine, are one of the most significant sources of polyphenols. Grapes, along with their skins, pulps, seeds, and stems, contain a considerable number of different phenolic compounds, mainly flavonols, non-flavonoids, and stilbenes.

For a long time, because of their health effects, the development of grape and wine production methods and their phenolic content research is being of interest. There are lots of studies showed that polyphenols, especially resveratrol and antioxidative phenolics, have a preventative effect for most diseases primarily, for cancer and cardiovascular disease (Lamuela-Raventós et al., 2001; He et al., 2008; Gómez-Serranillos and González-Burgos, 2013; Sancho and Mach, 2015; Briguglio et al., 2020). Flavonoids and stilbenes are potent antioxidants of red wine polyphenols that assisted human health without perceivable side effects and are involved in cancer protection (He et al., 2008). Their antioxidant and anti-inflammatory properties make resveratrol, epigallocatechin gallate, and curcumin among the most extensively studied polyphenols (Briguglio et al., 2020).

The development of phenolic compounds in the vineyard, extraction and modification of phenolic compounds during wine production and destiny of phenolic compounds during aging are the main areas of grape and wine phenolic research (Kennedy et al., 2006). The products formed during the processing of grapes to obtain wine and grape juice, and high-value food components grape by-products such as pomace and, stalks are rich sources of phenolic compounds and dietary fiber that could be used to obtain natural additives and nutraceuticals. The holistic use of grape by-products represents a successful opportunity to have economic benefits for agro-industrial activity, with a beneficial impact on the environment (Borman and Elder, 2018).

A significant number of studies were conducted on identifying and determining polyphenolic constituents in grape products using high-performance liquid chromatography (HPLC). However, as a consequence of the complication of the polyphenolic compound of grape products for example wine and the great structural variations of phenolics, some major phenolic compounds cannot be detected simultaneously in a single analysis, and their analysis takes a long time (Burin et al., 2011; Natividade et al., 2013).

Curiously, few approved literatures have been written upon an evaluation of polyphenolics by HPLC method (Natividade et al., 2013) validated method for the contemporaneous view of a few phenolics compounds in grape products (González-Barrio et al., 2009; Natividade et al., 2013). Few studies supplied data coupled with validating chromatographic methods for the associated scope (Sautter et al., 2005). Commonly, the validation procedures do not demonstrate all efficiency parameters required to evaluate the eligibility for a goal (Thompson et al., 2002; Chanda Gupta, 2015).

However, as far as we know, no appropriate, confirm analytical procedure has been described for the synchronous detection and quantification of 18 polyphenolics compounds. Hence, the objective of the present research was to enhance and confirm a procedure for the description and quantitation of the bioactive components in the grape matrix. As a result, a highly specific, sensitive, and simple chromatographic procedure was brought out and validated to determine 18 polyphenols, which pertain to the classes of flavonols, phenolic acids, etc, in grape juice, and red wines. HPLC was used to determine the phenolics with reversed-phase mode. This chromatographic program was optimized and validated by assessing the linearity, precision, accuracy.

Material and Method

Material and Chemicals

All the samples of red wines and fresh grapes were obtained from a nearby market and the grape juices were freshly juiced from the grapes. All solvents used were of chromatographical and analytical grade. The chemicals were supplied by Sigma-Aldrich (St. Louis, MO, USA). All the polyphenolic standards were of purity > 95%. Ultrapure water was acquired by a Milli-Q system (Millipore, Bedford, MA, USA).

Extraction of the Phenolics

Before analysis,100 mL of wine and grape juice samples were degassed with an ultrasonic bath (40 kHz) for 30 min and then filtered through a 0.45 µm nylon membrane. The volume of 1 mL of filtered wine and grape juice with 0.85% acetic acid solution was completed to 2.0 mL (VÁZquez-Armenta et al., 2018).

Apparatus

Shimadzu LC10A (Kyoto, Japan) chromatographic system was used as the HPLC system. This system consisted of an SPD-M10AVP diode array detector, LC-10 AD analytical pump, CTO10 column furnace, Rheodayn valve manual injector (7725i) and CBM-10A communication bus module providing data communication.

HPLC conditions

The analytical column, temperature gradient operating conditions and, etc. used for the determination of phenolic compounds could be seen in Table 1. The column effluent was monitored at 260, 280, 320, and 360 nm wavelengths for the information and data acquiring in all the PDA chromatograms. It was studied by scanning the wavelength range of 220-550 nm with a PDA detector. The molecular formula and weight, the maximum absorbance wavelength and the retention times of the standards were shown in Table 4.

Mobile Phase and Standard Solutions

Solvents and mobile phases to be used in chromatographic processes were degassed before being used and filtered through a 0.45 µm nylon membrane. All standard solutions used in calibration graphs and determination of other validation parameters were diluted from 500 mg L^{-1} stock solution. The stock solution of the standards was stored at -18°C until next use. Stock solutions of all standards (500 mg L^{-1}) were prepared in H_2O-MeOH (20:80 v/v). Working standards were freshly prepared by diluting the stock solution between 0.05 mgL^{-1} to 150 mgL^{-1}, concentrations in the same solvent. The calibration curve was assembled by plotting each standards' concentration against peak area. All solutions were stored at 4°C to their shelf life.

Method Validation

The proposed chromatographic program was validated for parameters such as accuracy, linearity, precision, etc. To raise the calibration curves, linearity was tested at three different concentrations of the polyphenols. The limits of detection (LOD) and quantification (LOQ) were estimated for each polyphenolic constituents. A corresponding standard solution was used based on the signal-to-nose ratio (S/N) of 3 and 10, respectively. Accuracy was evaluated by the standard addition method. Three different concentrated standard solutions (1, 20 and 40 mg L^{-1}) were added to the samples, and the recovery was evaluated in three replications for each fortification level. For comparison, untreated red wine and grape juice samples were also analyzed. Several parameters such as linearity, range, LOD, LOQ, accuracy, and precision of phenolics in wine and grape juice were assessed. The validation parameters specificity was considered based on current directives outlined by ICH and United States Pharmacopeia guidelines (Borman and Elder, 2017).

Table 1. Chromatographic Conditions for the Determination of Phenolic Compounds

Chromatographical variables	Chromatographic conditions
Injection volume	25 µL
Analytical column	ODS4 reverse-phase column, 4.6 × 250 mm, 5 µm particle size
Mobile phase	A (acetic acid in water, pH 2.00) B (20 % solution A and 80 % acetonitrile)
Gradient programme	First segment: 0.01–25 min: 0–35% B Second segment: 25–40 min: 35–60% B Third segment: 40–45 min: 60–100% B Conditioning step: 45–55 min: 100–0% B
Flow rate	1.0 mL/min
Temperature	35°C ± 1
Detection	260,280, 320, and 360 nm

Table 2. Assay validation parameters of the proposed HPLC method for determination of 18 polyphenolic compounds

Polyphenolic compounds		Precision		Linearity			R	LOD[c]	LOQ[c]
Compounds name	MFW	RP[a]	IP[b]	Slope	Intercept	CC (r^2)			
Gallic acid monohydrate (GA)	$C_7H_8O_6$(188.135)	±1.22	±2.00	191262	+12312	0.9996	0.5-150	0.22	0.715
(−)-Gallocatechin (GC)	$C_{15}H_{14}O_7$(306.267)	±1.89	±1.89	4824.98	-440.98	0.9968	0.5-150	0.250	0.800
Caffeic acid (CA)	$C_9H_8O_4$(180.157)	±2.01	±2.12	3768.32	-398.45	0.9993	0.25-150	0.085	0.275
Vanillic acid (VA)	$C_8H_8O_4$(168.147)	±1.75	±3.00	7147.50	+441,74	0.9941	0.05-120	0.044	0.146
Ellagic acid (EA)	$C_{14}H_6O_8$(302.193)	±2.21	±2.76	696.45	+112.34	0.9956	0.5-150	0.650	1.980
p-Coumaric acid glucoside (p-CA)	$C_{15}H_{18}O_8$(326.299)	±2.23	±1.81	128.95	+29.77	0.9995	0.25-150	0.085	0.280
Sinapinic acid (SA)	$C_{11}H_{12}O_5$(224.210)	±1.78	±1.96	712.34	-456,23	0.9975	0.5-150	0.486	0.998
trans-Ferulic acid (t-FA)	$C_{10}H_{10}O_4$(194.184)	±1.99	±2.03	387.95	-132.56	0.9990	0.15-155	0.065	0.200
Resveratrol (Res)	$C_{14}H_{12}O_3$(228.243)	±1.54	±1.76	416.87	-214.87	0.9973	0.15-150	0.045	0.147
Rutin trihydrate (Rut)	$C_{27}H_{36}O_{19}$(664.563)	±1.48	±2.23	1125.43	-1062.34	0.9987	0.5-150	0.180	0.590
(E)-p-Hydroxycinnamic acid (Hy-CinA)	$C_9H_8O_3$(164.158)	±1.75	±1.67	423.42	-579.21	0.9998	0.10-150	0.056	0.158
Chlorogenic acid (CGA)	$C_{16}H_{18}O_9$(354.309)	±1.63	±1.84	511.23	-17.97	0.9975	0.15-150	0.076	0.230
Kaempferol (Kae)	$C_{15}H_{10}O_6$(286.236)	±1.98	±2.08	38.45	-457.21	0.9951	0.10-100	0.040	0.125
(+)-Catechin (hydrate) (Cat)	$C_{15}H_{16}O_7$(308.283)	±1.48	±1.94	186.54	+28.45	0.9975	0.20-150	0.081	0.269
Protocatechuic acid	$C_7H_6O_4$(154.120)	±1.98	±2.12	138.49	-22.96	0.9935	0.25-125	0.175	0.525
Quercetin hydrate (Quar)	$C_{15}H_{12}O_8$(320.251)	±1.46	±2.25	398.74	-39.42	0.9968	0.15-150	0.077	0.257
Syringic acid (SA)	$C_9H_{10}O_5$(198.173)	±1.27	±1.48	896.28	+151.23	0.9964	0.10-100	0.032	0.152
(−)-Epicatechin-3-O-Gallate (Epi-C)	$C_{22}H_{18}O_{10}$(442.372)	±1.82	±2.59	119234	-1987	0.9901	0.05-100	0.030	0.105

[a]RP: Repeatability, The intraday (n = 3), an average of three concentrations (1.0, 20 and 40 mg L^{-1}) for compounds repeated three times within the day, [b] IP: Intermediate precision. The inter-day (n = 3), an average of three concentrations (1.0, 20 and 40 mg L^{-1}) for compounds repeated three times in 3 days, [c]Determined via calculations, LOD (mg L^{-1}) = 3.3 (SD of the response/slope), LOQ (mg L^{-1}) = 10 (SD of the response/slope), MFW: Molecular Formula and Weight, CC: Correlation coefficient, R: Range (mg L^{-1})

Determination of Linearity and Range

The linearity and range were determined using a mixture of standard solutions as an intermediate mixed standard solution. The linearity of the method was evaluated by recurrently injecting different concentrations of the standard solution of the intermediate mixed standard solution. The linearity and range were estimated by means of regression analysis from the calibration curve.

The range is the distance between the maximum and minimum grades of analyses that have been established. The range was determined with precision, accuracy, and linearity using the defined procedure.

Accuracy (Percentage Recovery)

The percentage of the polyphenols' recovery constitutes the accuracy value. To calculate the accuracy the blank sample (non-treated wine and grape juice samples) and three different concentrated (1, 20, and 40 mg L^{-1}) standard solution added wine and grape juice samples were analysed. The analyses were performed in triplicate, and five replicated were analysed at each fortification level. The recovery was calculated using Equation (1):

$$\text{Recovery } (\%) = \frac{\bar{x}' - \bar{x}}{x_{\text{contaminated}}} \times 100 \qquad (1)$$

where $\bar{x}$ is the average of analysis results with uncontaminated (unspiked) samples, $\bar{x}'$: Average of analysis results with contaminated (spiked) samples, $X_{contaminated}$ is amount of analyte used to contaminate (concentration added). The percentage of recovery of the standard solutions was measured as described in the literature (Marson et al., 2020).

LOD and LOQ

LOD was used to determine the sensitivity of the analytical method based on the visual detection method. The LOD was the minimum concentration of the detectable sample. Similar to the LOD, the limit of quantification was defined as a minimum quantity of analyte that can be quantitatively determined with proper accuracy and precision in the sampling. The LOD and LOQ were calculated from a benchmark of the measurement of the untreated sample with the analyte added sample (Marson et al., 2020; Rasool et al., 2020).

Determination of Precision

The precision was defined with two criteria, repeatability, and intermediate precision. The intermediate precision method was used within the intraday (n=3). Repeatability was calculated with an average of three concentrations (1.0, 20, and 40 mg L^{-1}) for compounds repeated three times within the day. Intermediate precision was an average of three concentrations (1.0, 20, and 40 mg L^{-1}) for compounds repeated three times in 3 days.

Statistical Analysis

The correlation coefficient analysis was performed by using the Minitab software (version 17 for PC, Minitab Inc., UK.). The slopes and intercepts of the calibration graphs were calculated by regressions.

Results and Discussion

The main purpose of our research was to improve and validate a highly specific, sensitive, and simple chromatographic methodology for the characterization and quantitation of the phenolic constituents in grape juice and red wine. The molecular structures of the analysed polyphenols were given in Figure 1.

Method Development

Preparatory studies were performed with C8 and C18-RP columns and various mobile phase compositions to select the correct column to determine 18 polyphenols in grape and red wine.

After the independent trials of different columns and gradient mobile phase, flow rates, running times and temperatures, the C18(ODS4-RP) column (4.6 × 250 mm, 5 µm particle size) with mobile phase of acetic acid in water (pH 2.00) and a mobile phase of 20% mobile phase and 80% acetonitrile, were chosen. For the best separation of the polyphenolics flow rate and temperature of the method were determined at 1.0 mL/min and 35°C ± 1, respectively. The detection wavelengths of 260, 280, 320, and 360 were chosen from the chromatograms of the standard solutions. The selected chromatographic conditions to determine phenolic compounds can be seen in Table 1. The chromatogram of the HPLC separation of 18 phenolic compounds with proven health effects, used as food supplements and nutraceuticals used in the food and pharmaceutical industry, is given in Figure 2.

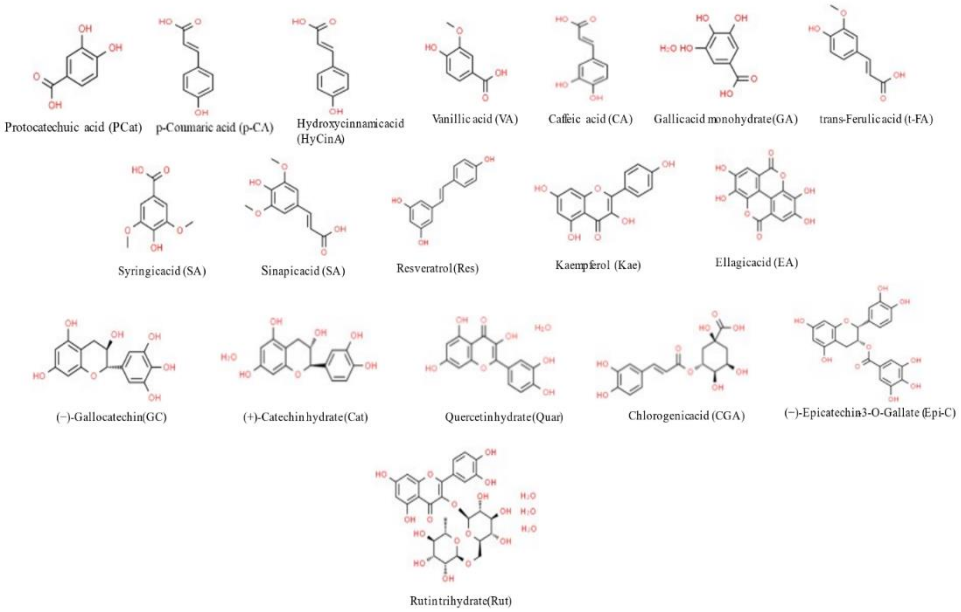

Figure 1. Structure of the 18 polyphenolics compound in grape matrix (sorted by molecular weight)

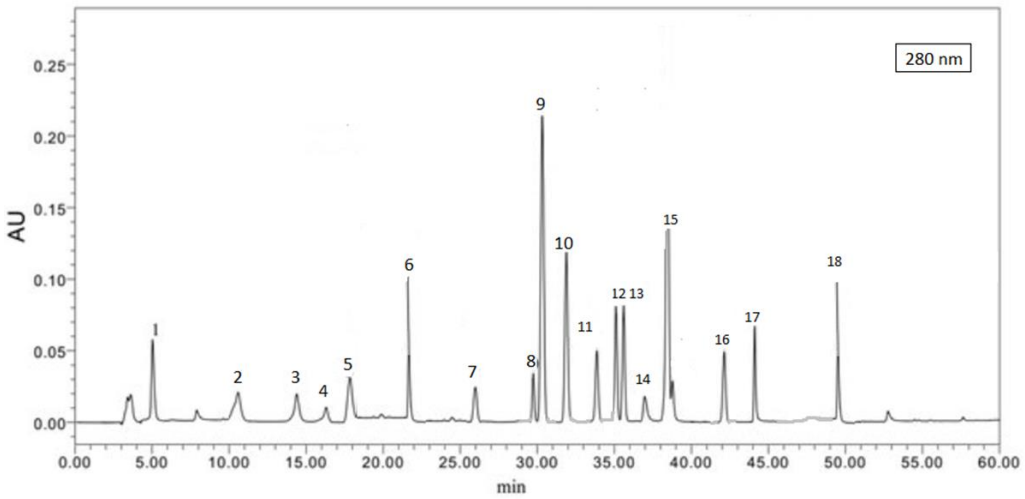

Figure 2. Typical chromatogram of 18 standard polyphenolics in standard solution mixtures using optimized chromatographic conditions: 1, Gallic acid monohydrate (GA); 2, Protocatechuic acid (pCat); 3, (−)-Gallocatechin (GC); 4, Caffeic acid (CA); 5, Vanillic acid (VA); 6, Ellagic acid (EA); 7, p-Coumaric acid (p-CA); 8, trans-Ferulic acid (t-FA); 9, Sinapic acid (SA); 10, Resveratrol (Res); 11, Rutin trihydrate (Rut); 12, (E)-p-Hydroxycinnamic acid (Hy-CinA); 13, Chlorogenic acid (CGA); 14, Kaempferol (Kae); 15, (+)-Catechin hydrate (Cat); 16, Quercetin hydrate (Quar); 17, Syringic acid (SA); 18,(−)-Epicatechin-3-O-Gallate (Epi-C)

Table 3. Determination of the method accuracy expressed as Recovery (%) from the red wine and grape juice samples with three different concentrations of the standard solution (Accuracy (%))

Compounds	MFW	Red wine		
		1.0 mg/L	20 mg/L	40.0 mg/L
Gallic acid monohydrate (GA)	$C_7H_8O_6$ (188.135)	105.58±5.48	93.44±1.86	97.65±0.89
Protocatechuic acid (PCat)	$C_7H_6O_4$ (154.120)	95.42±3.11	83.17±2.13	101.12±1.21
(−)-Gallocatechin (GC)	$C_{15}H_{14}O_7$ (306.267)	102.33±4.21	95.89±2.05	99.45±1.43
Caffeic acid (CA)	$C_9H_8O_4$ (180.157)	98.88±2.30	118.35±2.42	109.29±0.97
Vanillic acid (VA)	$C_8H_8O_4$ (168.147)	102.21±4.36	96.98±1.65	98.89±0.76
Ellagic acid (EA)	$C_{14}H_6O_8$ (302.193)	104.17±4.12	98.77±0.98	99.16±1.12
p-Coumaric acid (p-CA)	$C_9H_8O_3$ (164.158)	117.12±0.74	109.73±0.33	98.17±0.34
trans-Ferulic acid (t-FA)	$C_{10}H_{10}O_4$ (194.184)	115.09±0.75	105.98±0.89	97.86±0.65
Sinapic acid (SA)	$C_{11}H_{12}O_5$ (224.210)	97.23±3.76	89.87±1.76	95.45±0.73
Resveratrol (Res)	$C_{14}H_{12}O_3$ (228.243)	101.21±3.78	98.72±1.56	99.42±1.25
Rutin trihydrate (Rut)	$C_{27}H_{36}O_{19}$ (664.563)	99.12±4.23	98.25±4.82	101.21±1.43
(E)-p-Hydroxycinnamic acid (Hy-CinA)	$C_9H_8O_3$ (164.158)	102.87±4.56	98.65±2.54	99.87±2.11
Chlorogenic acid (CGA)	$C_{16}H_{18}O_9$ (354.309)	102.33±3.45	98.76±2.01	100.34±0.92
Kaempferol (Kae)	$C_{15}H_{10}O_6$ (286.236)	103.23±3.65	96.76±2.23	98.92±0.89
(+)-Catechin hydrate (Cat)	$C_{15}H_{16}O_7$ (308.283)	93.09±2.44	119.88±1.26	95.76±1.06
Quercetin hydrate (Quar)	$C_{15}H_{12}O_8$ (320.251)	112.32±4.49	97.78±2.21	98.92±1.16
Syringic acid (SA)	$C_9H_{10}O_5$ (198.173)	101.65±3.76	98.76±2.45	99.86±2.47
(−)-Epicatechin-3-O-Gallate (Epi-C)	$C_{22}H_{18}O_{10}$ (442.372)	99.87±3.54	89.98±2.23	97.76±0.93
Compounds	MFW	Grape juice		
		1.0 mg/L	20 mg/L	40.0 mg/L
Gallic acid monohydrate (GA)	$C_7H_8O_6$ (188.135)	101.23±3.87	98.23±1.02	95.18±0.78
Protocatechuic acid (PCat)	$C_7H_6O_4$ (154.120)	97.23±2.95	99.98±0.98	102.23±0.86
(−)-Gallocatechin (GC)	$C_{15}H_{14}O_7$ (306.267)	105.02±2.28	101.53±1.23	99.65±1.12
Caffeic acid (CA)	$C_9H_8O_4$ (180.157)	117.61±0.03	104.51±0.53	96.63±0,62
Vanillic acid (VA)	$C_8H_8O_4$ (168.147)	99.87±2.56	95.73±0.87	98.22±0.78
Ellagic acid (EA)	$C_{14}H_6O_8$ (302.193)	102.12±2.24	97.15±0.89	99.27±1.12
p-Coumaric acid (p-CA)	$C_9H_8O_3$ (164.158)	109.33±2.12	115.61±3.86	95.93±1.08
trans-Ferulic acid (t-FA)	$C_{10}H_{10}O_4$ (194.184)	114.2 1±1.98	117.83±2.03	102.44±1.89
Sinapic acid (SA)	$C_{11}H_{12}O_5$ (224.210)	102.54±2.65	97.98±1.65	99.87±1.21
Resveratrol (Res)	$C_{14}H_{12}O_3$ (228.243)	97.43±4.22	97.87±1.78	95.43±0.87
Rutin trihydrate (Rut)	$C_{27}H_{36}O_{19}$ (664.563)	102.67±2.98	99.65±2.87	98.65±1.22
(E)-p-Hydroxycinnamic acid (Hy-CinA)	$C_9H_8O_3$ (164.158)	102.35±3.87	97.23±1.87	97.43±0.86
Chlorogenic acid (CGA)	$C_{16}H_{18}O_9$ (354.309)	99.65±2.21	99.45±0.89	97.99±1.23
Kaempferol (Kae)	$C_{15}H_{10}O_6$ (286.236)	99.87±2.87	100.21±0.98	98.97±1.43
(+)-Catechin hydrate (Cat)	$C_{15}H_{16}O_7$ (308.283)	108.94±1.93	88.20±0.33	97.54±0.82
Quercetin hydrate (Quar)	$C_{15}H_{12}O_8$ (320.251)	99.87±3.32	100.05±0.87	98.76±1.27
Syringic acid (SA)	$C_9H_{10}O_5$ (198.173)	98.46±2.87	101.54±0.87	99.78±1.00
(−)-Epicatechin-3-O-Gallate (Epi-C)	$C_{22}H_{18}O_{10}$ (442.372)	102.76±3.12	98.98±1.32	101.11±0.94

The accuracy average of (n = 3). Analytical results are the average of triplicates (mean ± sd), MFW: Molecular Formula, and Weight,

Table 4. Precision of the Method According to Retention Time (tR) and Average Concentration of the Phenolic Compounds (mg L^{-1}) in Red Wine, and Grape Juice Sample

Compounds	Molecular Formula and Weight	Abs (nm)	t_R min (SD)	Red Wine		Grape Juice	
				Average Conc. (SD)	RSD %	Average Conc. (SD)	RSD %
Gallic acid monohydrate (GA)	$C_7H_8O_6$ (188.135)	280	5.15 (0.34)	32.45(0.25)	1.22	5.05(0.98)	1.73
Protocatechuic acid (PCat)	$C_7H_6O_4$ (154.120)	280	10.10 (0.89)	7.45(0.07)	1.59	2.56(0.05)	1.97
(−)-Gallocatechin (GC)	$C_{15}H_{14}O_7$ (306.267)	280	15.95 (1.06)	8.15(0.08)	1.78	0.57(0.10)	1.01
Caffeic acid (CA)	$C_9H_8O_4$ (180.157)	320	16.49 (1.23)	7.89(0.04)	1.65	3.48(0.05)	1.95
Vanillic acid (VA)	$C_8H_8O_4$ (168.147)	260	17.45 (1.34)	4.55(0.02)	1.87	0.16(0.05)	1.89
Ellagic acid (EA)	$C_{14}H_6O_8$ (302.193)	280	22.54 (1.87)	10.51(0.40)	2.02	0.38(0.05)	1.93
p-Coumaric acid (p-CA)	$C_9H_8O_3$ (164.158)	290	25.60 (1.97)	4.01(0.65)	1.43	5.24(0.97)	1.96
trans-Ferulic acid (t-FA)	$C_{10}H_{10}O_4$ (194.184)	280	29.89 (2.04)	12.54(0.09)	1.65	0.16(0.05)	1.86
Sinapic acid (SA)	$C_{11}H_{12}O_5$ (224.210)	320	30.71 (2.34)	1.02(0.04)	1.27	0.28(0.05)	1.77
Resveratrol (Res)	$C_{14}H_{12}O_3$ (228.243)	320	31.54 (1.97)	18.25(0.74)	1.54	0.67(0.05)	1.39
Rutin trihydrate (Rut)	$C_{27}H_{36}O_{19}$ (664.563)	260	33.99 (2.21)	8.49(0.12)	1.85	1.98(0.27)	1.65
(E)-p-Hydroxycinnamic acid (Hy-CinA)	$C_9H_8O_3$ (164.158)	280	35.98 (1.76)	2.25(0.06)	1.48	0.31(0.08)	1.89
Chlorogenic acid (CGA)	$C_{16}H_{18}O_9$ (354.309)	320	36.66 (0.98)	0.5(0.02)	1.66	2.26(0.43)	1.79
Kaempferol (Kae)	$C_{15}H_{10}O_6$ (286.236)	280	37.45 (0.96)	16.21(0.35)	1.92	1.45(0.33)	1.90
(+)-Catechin hydrate (Cat)	$C_{15}H_{16}O_7$ (308.283)	280	38.15 (1.05)	102.14(1.05)	1.41	25.98(1.58)	2.11
Quercetin hydrate (Quar)	$C_{15}H_{12}O_8$ (320.251)	360	41.92 (1.14)	2.37(0.01)	1.72	1.01(0.35)	2.56
Syringic acid (SA)	$C_9H_{10}O_5$ (198.173)	320	43.45 (2.27)	5.12(0.02)	1.57	2.20(0.05)	1.25
(−)-Epicatechin-3-O-Gallate (Epi-C)	$C_{22}H_{18}O_{10}$ (442.372)	280	49.85 (1.44)	5.45(0.06)	1.99	1.98(0.44)	2.21

Method Validation

After the best method parameters were selected and the method was developed, the validation; was carried through with linearity, precision, accuracy, and etc. parameters.

Linearity and Range

Linearity is the accomplishment of a method to reveal test results that are straightly commensurate to the analyte concentration within a given range (Marson et al., 2020; Rasool et al., 2020).

The evaluation of the method's linearity was assessed by the calibration curves of different concentrated standard solutions. The slope values of the linear calibration curve, the intercept values, the correlation coefficients, and the range values of the polyphenols were found in Table 2. Correlation coefficients of each polyphenol were above 0.99, displaying good linearity. The highest r^2 value was measured at (E)-p-Hydroxycinnamic acid (Hy-CinA) (r^2 =0.9998), while the lowest was at (−)-Epicatechin-3-O-Gallate (Epi-C) (r^2 =0.9901).

Al-Rimavi (2014) was remarked at his work that the correlation coefficient's (r^2) being not less than 0.99 was the acceptance criteria for the linearity (Al-Rimawi, 2014). All results of the correlation coefficients in our study satisfy the acceptance conditions in line with the work of Al-Rimavi and the other literature (Burin et al., 2011; Al-Rimawi, 2014; Restivo et al., 2014).

Accuracy (Percentage Recovery)

The accuracy was calculated by the recovery values of untreated samples, and the certain amount of polyphenols added samples values within the calibration range. The accuracy was measured at three concentration levels (1, 20 and 40 mg L^{-1}) by using known quantities of phenolic content. The recovery of polyphenolic constituents was ranged from 83.17 % to 119.88 % for red wine and 88.20 % to 117.83 % for grape juice. The accuracy results were present in Table 3. The results have demonstrated that the developed method has a good recovery for selected polyphenols at chosen concentration levels.

Precision

The retention time and the mean polyphenol concentration denoted as relative standard deviation (%RSD), was formed the basis for the precision of this method. Results for repeatability and intermediate precision were shown in Table 2. For all grape product matrices in all three concentration levels and intermediate precision, a %RSD below 3.0% was obtained. In general, the values were similar to the %RSD limit defined by the other authors (Burin et al., 2011; Natividade et al. 2013).

LOD and LOQ

Table 2 indicated the LOD and LOQ values. The LOD values ranged from 0.030 to 0.650 mg/L, while LOQ values changed between 0.105 to 1.980 mg/L. According to the results, it can be seen that the LOQ values were almost three times higher than the LOD values. These results were in agreement with the literature data (Burin et al., 2011). This result confirms that the proposed method is appropriated and sensitive enough for the determination and quantification of selected phenolic constituents in red wine and fruit juice, even in low concentration levels.

Conclusion

The stated purpose of this study was to improve and validate an analytical method for the recognition and quantification of 18 polyphenolic compounds with proven health effects found in red wine and grape juice. As a result, a highly specific, sensitive, and simple chromatographic method has been presented and validated. The method meets the purpose of the study. Moreover, the most important challenge at the phenolic research was detecting some major phenolic compounds simultaneously in a single analysis in a short time. The running time of 55 minutes to detect 18 polyphenols in a sample became a solution for this challenge. Additionally to a short analysis time, the developed method has the advantages of eliminating the complex sample extraction and sample preparation, exhibiting great precision, accuracy, and linearity for the detection of the polyphenols in wine and grape juice.

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