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Received November 29, 2009
Revised January 12, 2010
Accepted January 13, 2010

Research Article

HPLC-DAD methodology for the quantification of organic acids, furans and polyphenols by direct injection of wine samples

This article proposes a simple and sensitive HPLC method with photo-diode array detection for the analysis of organic acids, monomeric polyphenols and furanic compounds in wine samples by direct injection. The chromatographic separation of 8 organic acids, 2 furans and 22 phenolic compounds was carried out with a buffered solution (pH 2.70) and acetonitrile as mobile phases and a difunctionally bonded C18 stationary phase, Atlantis dC18 (250×4.6 mm, 5 μm) column. The elution was performed in 12 min for the organic acids and in 60 min for the phenolic compounds, including phenolic acids, stilbenes and flavonoids. Target compounds were detected at 210 nm (organic acids, flavan-3-ols and benzoic acids), 254 nm (ellagic acid), 280 nm (furans and cinnamic acid), 315 nm (hydroxycinnamic acids and *trans*-resveratrol) and 360 nm (flavonoids). The RSD for the repeatability test ($n = 5$) of peak area and retention times were below 3.1 and 0.3%, respectively, for phenolics and below 1.0 and 0.2% for organic acids. The RSDs expressing the reproducibility of the method were higher than for the repeatability results but all below 9.0%. Method accuracy was evaluated by the recovery results, with averaged values between 80 and 104% for polyphenols and 97–105% for organic acids. The calibration curves, obtained by triplicate injection of standard solutions, showed good linearity with regression coefficients higher than 0.9982 for polyphenols and 0.9997 for organic acids. The LOD was in the range of 0.07–0.49 mg/L for polyphenols (cinnamic and gallic acids, respectively) and 0.001–0.046 g/L for organic acids (oxalic and lactic acids, respectively). The method was successfully used to measure and assess the polyphenolic fingerprint and organic acids profile of red, white, rosé and fortified wines.

Keywords: Direct injection / Furans / HPLC method with photo-diode array detection / Organic acids / Polyphenols
DOI 10.1002/jssc.200900784

1 Introduction

Analytical characterization of wines is usually a time-consuming process, but it yields the necessary information for the elaboration and control of a quality product and definition of suitable conditions for adequate preservation. The profile and evaluation of the organic acids and polyphenols content are important parameters in wineries, and hence it is essential to have a rapid and precise methodology for quantification. The determination of organic acids, mainly tartaric, malic and lactic acids, is important for the fermentation process monitoring, as they contribute to flavour balance, chemical stability and microbiologic control and frequently subject to control in food to accomplish law and regulations. In addition, polyphenols also have effects on the organoleptic characteristics (colour, flavour and taste), thus their profile and content are also significant [1, 2]. These two types of chemical species are very common in wines and both are affected by several factors such as ripening, variety, growing region, atmospheric conditions as well as production techniques [3–6].

The most frequent acids found in wines are tartaric, malic and citric acids originated from the grape, and succinic, lactic and acetic acids resulting from alcoholic and malolactic fermentations. Acetic acid can also increase during ageing period. Eventually, other acids can occur in

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Abbreviations: HMF, 5-hydroxymethylfurfural; **HPLC-DAD,** HPLC with photo-diode array detection

small amounts which may be derived from ethanol oxidation [7]. One of the most used technologies to detect and quantify organic acids is HPLC method with photo-diode array detection (HPLC-DAD) and there are a number of published methods [8–13], some methods are based on ion-exclusion separations [12, 14], which normally require the removal of polyphenols before sample analysis, others involve ion-exchange [15] and RP separations [16–18]. Most methods are not applicable to wine due to the alcohol content or low resolution [19]. Frequently, isocratic elutions are described using an acidified aqueous solvent and separation time is no longer than 20 min [20]. It is also common to find in the literature that organic acid analysis includes a sample pretreatment which increases analysis time and affects the reliability of the results.

From the enological point of view, phenolic compounds mainly influence the colour, astringency, bitterness, clarity as well as the browning process [21–23]. Besides of their enological attributes, polyphenols are known to potentiate some health benefits effects due to their pharmacological activities, such as antioxidant, anti-inflammatory, anti-allergic, antiviral, anticarcinogenic, antimicrobial and vasodilatory actions [24–27]. Phenolic acids, stilbenes, flavanols and anthocyanins are the main types of polyphenols present in wines. Some examples which are frequently reported are gallic acid, ferulic acid, quercetin, myricetin, catechin, epicatechin and *trans*-resveratrol [28–30]. A variety of techniques have been used for the determination of phenolic compounds in wines based on GC [31–34] and CE [35–37], but RP-HPLC has been elected and considered the most appropriate technique to analyze wine polyphenols, often used to give product composition and differentiation [38–40]. Generally, studies make use of RP C18 columns [41, 42] and binary solvent systems consisting of a solvent A, usually acidified water, and a polar organic solvent B, such as acetonitrile or methanol [43]. DAD methods are the most common [11–13, 38, 40, 41, 44–56], but other detection methods as electrochemical [57, 58] and MS [59–61] have also been used. The use of LC-MS and LC-MS/MS has become the best option for the analysis of these compounds in several matrices as well as their derived products [62–64], but the opportunity of access to these advanced technologies is still restricted for most laboratories. In Table 1, several published methods are summarized for the determination of these compounds in wine and similar matrices.

Therefore, the aim of this study was to develop a simple and sensitive methodology using RP-HPLC-DAD chromatographic separation, allowing a single run determination of organic acids and monomeric polyphenols in the same wine sample, with no sample pretreatment, covering the compounds normally found in wines. RP separation mechanism was chosen since it is frequent in polyphenol analysis and performs organic acids faster analysis [65]. Other HPLC procedures have also been developed for the simultaneous analysis of organic acids and polyphenols in wines and grapes [11, 66], but these studies were developed for a restricted number of polyphenolic compounds. For the purpose of the study, the method was extended to two furanic compounds, 5-hydroxymethylfurfural (HMF) and furfural, as they are usually detected in fortified wines. Considering the elution conditions, both furans are presented in tables associated with polyphenols. The current project intends to apply the developed methodology for the assessment of these compounds in several wine types: fortified, red, white and rosé wines.

2 Materials and methods

2.1 Standards and reagents

Polyphenol standards: gallic acid, gentisic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, cinnamic acid, *p*-hydroxybenzoic acid, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, myricetin, sinapic acid, rutin and kaempferol were supplied by Fluka Biochemika AG (Buchs, Switzerland), protocatechuic acid, vanillin, syringic acid and *trans*-resveratrol by Sigma-Aldrich (St. Louis, MO, USA), whereas syringaldehyde, HMF and furfural were acquired from Acros Organics (Geel, Belgium) and quercetin from Riedel-de-Haën (Seelze, Germany). The purity of all polyphenolic standards was greater than 95%. Polyphenol stock solutions of 1 g/L were prepared by dissolving the appropriate amount of each compound in ethanol. These solutions were stored at 4°C and diluted before use with Milli-Q water to prepare the working standard solutions.

Acids standards were obtained from different suppliers: L-tartaric (99.5%), L-malic (99.5%) and succinic (99.5%) from Merck (Darmstadt, Germany); lactic (85%) and acetic (99.7%) from Panreac Química S.A. (Barcelona, Spain); citric (99.5%) from Fluka BioChemika AG; formic (99.7%) and oxalic (99%) were obtained from Fisher Scientific (Loughborough, UK) and Acros Organics, respectively. Stock standard solutions of 10 g/L were prepared by dissolving each acid in Milli-Q water and stored at 4°C for 1 month. Working standard solutions were prepared by dilution with Milli-Q water.

HPLC-grade acetonitrile was obtained from Sigma-Aldrich and ultra-pure water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). Disodium hydrogen phosphate dihydrate (99%) was supplied by Panreac Química S.A., sulfuric acid (95–97%) was supplied by Riedel-de-Haën. The eluents were previously filtered with membrane filters obtained from Pall (0.20 μm, Ann Arbor, MI, USA).

2.2 Apparatus and operating conditions

Chromatographic analyses were carried out using a Waters Alliance liquid chromatograph (Milford, MA, USA)

Table 1. HPLC-DAD methods reported in the literature for the analysis of polyphenols and organic acids in wines and/or similar matrices[a)]

Samples	Analytes	Stationary phases	Eluents	Detection wavelenghts (nm)	LOD (mg/L)	Ref.
Red wines LLE	15 polyphenols	ODS-Hypersil (2.1 id × 100 mm, 5 μm), $T = 40°C$	Gradient: A: acidified water (0.6% perchloric acid); B: methanol; Flow: 0.3 mL/min	280		[44]
Red wines DI	20 polyphenols including anthocyanins	LiChrospher RP-18 (4.0 id × 250 mm, 5 μm), $T = 40°C$	Gradient: A: 9 mM aqueous orthopho sphoric acid, pH 2.5; B: solvent A/acetonitrile, 75:25 v/v; Flow: 1.0 mL/min	280, 320, 360 and 520		[45]
Red wines SPE	12 polyphenols	Hypersil ODS (4.6 id × 200 mm, 5 μm)	Gradient: A: acetic acid in water, 2% v/v; B: water/acetonitrile/acetic acid, 78:20:2 v/v/v; Flow: 1.0 mL/min	254, 280 and 340	0.05–1.95	[49]
Red wines LLE	16 polyphenols	ODS-Hypersil (2.1 id × 200 mm, 5 μm)	Gradient: A: water/formic acid, 99:1 v/v; B: methanol; Flow: 0.3 mL/min	280, 320 and 350		[40]
Red wines DI	35 polyphenols including anthocyanins	Spherisorb C18 (4.6 id × 250 mm, 5 μm), $T = 40°C$	Gradient: A: 50 mM aqueous ammonium hydrogenphosphate, pH 2.6; B: solvent A/ acetonitrile, 20:80 v/v; C: 200 mM phosphoric acid, pH 1.5	280, 320, 360 and 520		[50]
Red wines LLE	47 polyphenols	Nova-Pak C18 (3.9 id × 300 mm, 4 μm)	Gradient: A: acetic acid in water, 2% v/v; B: water/acetonitrile/acetic acid, 78:20:2 v/v/v; Flow: 0.7 mL/min	280, 340 and 310		[51]
Red wines DI	30 polyphenols including anthocyanins	Atlantis dC18 (2.1 id × 250 mm, 5 μm), $T = 30°C$	Gradient: A: formic acid in water, 5% v/v; B: acetonitrile/ water/formic acid, 80:15:5 v/v/v; Flow: 0.25 mL/min	280, 320, 360 and 520 nm		[56]
Red wines SPE	6 organic acids	Nucleogel ion 300 OA (7.7 × 300 mm), $T = 30°C$	Isocratic: 0.01 N sulfuric acid; Flow: 0.2 mL/min	214	0.01–1.67	[12]
Red wines DI for hydroxycinnamic acids	38 polyphenols including anthocyanins	Waters symmetry C18 (4.6 id × 150 mm, 5 μm), $T = 35°C$	Gradient: A: formic acid in water, 5% v/v; B: methanol; Flow: 1.0 mL/min	280, 320, 360 and 520		
LLE followed by SPE for hydroxybenzoic acids, catechins and flavonols		ODS Hypersyl (4.6 id × 250 mm, 5 μm), $T = 35°C$	Gradient: A: formic acid in water, 2.5% v/v; B: methanol; Flow: 1.0 mL/min			[38]
Red wines DI	48 polyphenols including anthocyanins	Ace® 5 C18 (4.6 i.d × 250 mm), $T = 20°C$	Gradient: A: 50 mM aqueous ammonium hydrogenphosphate, pH 2.6; B: solvent A/acetonitrile, 20:80 v/v; C: 200 mM phosphoric acid, pH 1.5	280, 320, 360 and 520	0.088–0.711	[53]
Red wines DI	6 polyphenols	LC18 RP packing (Supelco) (2.1 id × 150 mm, 5 μm)	Gradient: A: 5% formic acid in water; B: acetonitrile; Flow: 0.3–0.8 mL/min in 7 min	285, 306 and 270	0.16–1.50	[54]
Red wines SPE for organic acids	11 polyphenols and 2 organic acids	LichroCART® 250–4 Superspher® RP 18	Isocratic: 5 mM phosphoric acid; Flow: 0.7 mL/min	210		[13]

Table 1. Continued

Samples	Analytes	Stationary phases	Eluents	Detection wavelenghts (nm)	LOD (mg/L)	Ref.
		(4.6 id × 250 mm, 5 μm)				
LLE for polyphenols		Superpher® 100, C18 (4.6 id × 250 mm, 5 μm)	Gradient: A: water/acetic acid, 98:2 v/v; B: water/methanol/acetic acid, 68:30:2 v/v/v; Flow: 1.0 mL/min	254, 280 and 320		
Musts and wines from red grapes						
DI	7 organic acids 6 polyphenols	Synergi™ Polar-RP™ (4.6 id × 250 mm), $T = 30^{\circ}C$	Gradient: A: trifluoroacetic acid in water, 0.2% v/v, pH 1.9; B: acetonitrile; Flow: 1.5 mL/min	210 and 280		[11]
White wines						
DI	17 polyphenols	Nova-Pak C18 (3.9 id × 300 mm, 4 μm), $T = 20^{\circ}C$	Gradient: A: acetic acid in water, 2% v/v; B: water/acetonitrile/acetic acid, 58:40:2 v/v/v; Flow: 1.0 mL/min	280 and 320		[52]
White grapes and their juices						
DI	3 organic acids	Bio-Rad Aminex HPX-87 (300 × 7.8 mm)	Isocratic: 0.01 N sulfuric acid; Flow: 0.6 mL/min	214	15.0–30.0	[10]
Red and white wines						
DI	17 polyphenols	Chromolith Perfor mance RP-18e (4.6 id × 100 mm), $T = 30^{\circ}C$	Gradient: A: methanol/double-distilled water, 2.5:97.5 v/v, at pH 3 with H_3PO_4; B: methanol/double-distilled water, 50:50 v/v, at pH 3 with H_3PO_4; Flow: 1.0 mL/min	256, 280, 308, 324 and 365	0.010–0.160	[46]
Red and white wines						
SS-LLE	13 polyphenols	Agilent Zorbax Eclipse XDB-C18 (4.6 id × 250 mm, 5 μm)	Gradient: A: water/methanol/formic acid, 97:2.5:0.5 v/v/v; B: methanol; Flow: 1.0 mL/min	280, 305 and 370	0.073–0.164	[41]
Red, white and rosé wines						
LLE	17 polyphenols	Nova-Pak C18 (3.9 id × 150 mm, 4 μm)	Gradient: A: water/acetic acid/methanol, 88:2:10 v/v/v; B: water/acetic acid/ methanol, 8:2:90 v/v/v; Flow: 0.7 mL/min	270, 307 and 360	0.03–11.5	[55]
Musts and fortified wines						
SPE followed by NBDI derivatization	6 organic acids	Spherisorb C18 (4.6 id × 150 mm, 3 μm)	Gradient: A: water; B: acetonitrile; Flow: 1.5 mL/min	265	5.0–98.0	[9]
Wines						
LLE	16 polyphenols	Phenomenex Luna C18 (4.6 id × 150 mm, 5 μm)	Gradient: A: formic acid in water, 0.1% v/v; B: methanol; Flow: 0.7 mL/min	λ with lowest energy (λ_{max})	0.01–0.03	[48]
Brandies						
DI	13 polyphenols	Lichrospher RP18 (4.0 id × 250 mm, 5 μm), $T = 40^{\circ}C$	Gradient: A: formic acid in water, 2% v/v; B: methanol/water/formic acid, 70:28:2 v/v/v; Flow: 1.0 mL/min	280 and 320	0.01−1.15	[47]

a) LLE, liquid–liquid extraction; DI, direct injection; SS-LLE, solid-supported liquid–liquid extraction.

equipped with an auto-injector (Waters 2695, separations module) and a photodiode array detector (Waters 2996). To separate organic acids and polyphenols, an Atlantis dC18 column (250 mm × 4.6 mm id; 5 μm; Milford, MA, USA) was selected as the analytical column, using the following mobile phases: A: 10 mM of phosphate solution buffered at pH 2.70 with concentrated sulphuric acid; B: 100% acetonitrile.

As polyphenols are present in wine in minor quantities (about mg/L) when compared with organic acids content (up to g/L), the separation method was divided into two steps, maintaining the general operation conditions but allowing the correct evaluation of the different concentration ranges. Organic acids chromatographic separation was carried out using an isocratic elution, 100% A during 8 min followed by 12 min of washing and re-equilibration period, while polyphenols and the two furans require a gradient elution applied as follows: 0–30 min, 0–20% B, linear; 30–50 min, 20–50% B, linear; 50–60 min, washing and re-equilibration of the column. The mobile phase was set to a flow rate of 1.0 mL/min and the column thermostated at 30°C. Injection volume was set to 10 μL and all standards

and wine samples were injected in triplicate, after being filtered through membrane filters Acrodisc® CR PTFE from Waters (0.45 μm). Target compounds were detected at 210 nm (organic acids, flavan-3-ols and benzoic acids), 254 nm (ellagic acid), 280 nm (furans and cinnamic acid), 315 nm (hydroxycinnamic acids and *trans*-resveratrol) and 360 nm (flavonoids). The detector signals were recorded on a chromatography data system controlled by the Empower Pro software. Chromatographic peaks were identified by comparison of elution order, retention times, the spectral UV–Vis with those of standards and spiking samples with pure compounds. The quantification of the studied compounds was carried out using the external standard method.

2.3 Samples

This methodology was applied to different types of wines: four fortified wines (F wines), four red table wines (R wines), four white table wines (W wines) and one rosé wine (Rs wine). All wines were produced from *Vitis vinifera* L. grape varieties. Red and white wines were bought in local stores and fortified wines were supplied by a local producer. Samples were filtered (0.45 μm) and diluted with mobile phase A when needed to comply with the working range.

2.4 Method validation

Retention times were previously determined using individual standards dissolved in mobile phase A. The working range for each compound was estimated from the expected results for this type of samples and the higher concentration working standard solution was accordingly prepared from the stock solution of each compound (10 g/L for organic acids and 1 g/L for polyphenolic and furanic compounds) and diluted with Milli-Q water. Five other working solutions were prepared by successive dilutions and injected for the linearity range test.

Wide concentration ranges were used as the amount of the studied compounds depends on the wine variety. Quantification was carried out by the external standard method based on peak areas of the eluted compounds.

Method sensitivity was assessed by the determination of LOD and LOQ of each compound. These parameters were calculated on the basis of linear regression, $LOD = 3.3\sigma/b$ and $LOQ = 10\sigma/b$, σ is the y-intercept standard deviation and b is the slope of the linear regression.

The precision was evaluated by inter- and intra-day repetition method. Intra-day repeatability was assessed by five successive replicate determinations of three standards. Inter-day reproducibility was assessed by analyzing, on three distinct occurrences, five replicates of three standards.

Recovery was determined by the addition of known amounts of organic acids, furans and polyphenols to the wine samples, tested for two concentration levels and replicated three times. Average recovery was calculated by comparing mean values of replicates with theoretical concentrations of each replicate.

3 Results and discussion

3.1 Method development

Usually, the chromatographic analysis of organic acids is carried out using ion-exchange columns, requiring phenolic compounds to be previously removed from the sample, whereas the polyphenol separation is frequently performed by RP. The present method was developed to allow the sequential analysis of 8 organic acids, 22 monomeric phenolic and 2 furanic compounds commonly found in wines (Table 2), using the same RP column, a difunctional-bonded C18 stationary phase, Atlantis dC18 column.

Initial HPLC working conditions were selected based on the organic acids method published in Waters application notebook for Atlantis columns [67]. Then, the method was optimized in order to achieve good resolution for the maximum number of peaks in the shortest analysis time, considering the following parameters: injection volume, wavelength detection, the solvents used and the elution program. As summarized in Table 1, the separation of polyphenols usually involves the use of acid additives, aiming to suppress ionization, namely acetic and formic acids. Besides being target compounds, these additives absorb at 210 nm, affecting the use of this wavelength in the measurement of polyphenols, namely flavan-3-ols, which have higher absorptivity at 210 nm than at 280 nm. Avoiding the use of these acid additives, the alternative was the use of buffered mobile phase for acid pH adjustment. The initial concentration of the buffered mobile phase (20 mM) was decreased to 10 mM to avoid problems with precipitation and the abrasive affect of phosphate buffers on pump seals, but ensuring pH control. As phosphate buffers higher then pH 7 are known to accelerate the dissolution of silica and shorten severely the lifetime of silica-based HPLC columns, the resolution degradation was monitored and the column seemed to be unaffected at the low pH used in this method (2.70). The method was developed with the intention of simultaneous analysis of organic acids and polyphenols, in a single run, but for calibration purposes and considering their disproportionate concentration ranges in wines, it was preferred to perform their analysis separately. However, as organic acids elute at low retention times (up to 9 min) and furans and polyphenols elute at higher retention times, a single run analysis can be carried out without losing separation. Therefore, an isocratic elution was carried out for organic acids with the buffered mobile phase at pH 2.70 (Fig. 1) and a gradient elution was used for monomeric polyphenols and furans. The gradient elution, described in Section 2, was performed during 60 min, including washing and re-equilibration stage, starting with 100% of aqueous

Table 2. Retention times, peak identification, spectral bands (λ_{max}, in bold), detection wavelength ($\lambda_{detection}$) and linearity parameters of organic acids, furans and polyphenols obtained using the proposed methodology

#	t_R (min)	Compound	Chemical family	UV bands (nm)	$\lambda_{detection}$ (nm)	Linear range	a[a)]	b[b)]	R^2	LOD	LOQ	Recovery (%)
						g/L						
1	3.06	Oxalic acid	Organic acid	199	210	0.012–0.307	−25 918	7 060 916	0.9999	0.001	0.003	105
2	3.48	Tartaric acid	Organic acid	198	210	0.060–1.512	−7230	954 374	0.9997	0.010	0.031	97
3	3.71	Formic acid	Organic acid	200	210	0.120–3.001	−11 242	562 545	0.9997	0.021	0.064	104
4	4.33	Malic acid	Organic acid	198	210	0.122–3.045	−7765	489 490	0.9998	0.017	0.052	100
5	5.08	Lactic acid	Organic acid	198	210	0.239–5.976	−3134	133 420	0.9997	0.046	0.138	103
6	5.37	Acetic acid	Organic acid	200	210	0.239–5.985	−4889	151 930	0.9998	0.042	0.127	102
7	7.03	Citric acid	Organic acid	197	210	0.090–2.252	−9930	644 315	0.9998	0.012	0.037	105
8	8.61	Succinic acid	Organic acid	208	210	0.062–1.542	−14 435	1 262 365	0.9998	0.008	0.024	100
						mg/L						
9	12.40	Gallic acid	Hydroxybenzoic acid	216, 271	210	2.70–54.00	−51 608	77 647	0.9995	0.487	1.477	95
10	13.48	HMF	Furan	226, 284	280	1.50–30.00	−31 960	92 726	0.9995	0.271	0.821	93
11	15.01	Furfural	Furan	228, 277	280	0.75–15.00	−20 602	82 660	0.9986	0.229	0.694	82
12	17.33	Protocatechuic acid	Hydroxybenzoic acid	205, 219, 259, 293	210	0.80–15.90	−17 951	66 943	0.9993	0.176	0.534	80
13	21.35	Gentisic acid	Hydroxybenzoic acid	210, 324	210	0.80–16.05	—26 461	86 293	0.9992	0.193	0.585	81
14	22.46	*p*-Hydroxybenzoic acid	Hydroxybenzoic acid	196, 254	210	0.75–15.00	−17 117	61 694	0.9996	0.129	0.391	83
15	23.15	(−)-Epigallocatechin	Flavan-3-ol	206, 271	210	0.75–15.00	−26 412	107 423	0.9983	0.257	0.779	89
16	24.51	(+)-Catechin	Flavan-3-ol	203, 279	210	0.75–15.00	−22 836	95 136	0.9993	0.166	0.502	101
17	25.60	Vanillic acid	Hydroxybenzoic acid	208, 218, 260, 292	210	0.79–15.75	−16 325	56 327	0.9996	0.131	0.397	104
18	26.99	Caffeic acid	Hydroxycinnamic acid	218, 238, 324	315	0.84–16.80	−11 537	52 286	0.9996	0.142	0.430	90
19	27.49	Syringic acid	Hydroxybenzoic acid	217, 274	210	0.75–15.00	−17 089	71 916	0.9995	0.147	0.446	91
20	28.46	(−)-Epicatechin	Flavan-3-ol	203, 279	210	0.80–16.05	−21 504	94 208	0.9994	0.168	0.510	96
21	29.77	Vanillin	Hydroxybenzaldehyde	204, 230, 279, 307	210	0.75–15.00	−16 877	48 269	0.9996	0.123	0.374	98
22	32.22	Syringaldehyde	Hydroxybenzaldehyde	216, 307	210	0.78–15.60	−24 925	56 216	0.9982	0.272	0.824	102
23	32.86	*p*-Coumaric acid	Hydroxycinnamic acid	212, 226, 310	315	0.79–15.75	−14 506	73 759	0.9994	0.155	0.471	88
24	35.29	Ferulic acid	Hydroxycinnamic acid	217, 234, 323	315	0.79–15.75	−10 514	51 221	0.9996	0.141	0.426	92
25	35.91	Sinapic acid	Hydroxycinnamic acid	200, 237, 323	315	0.77–15.30	−10 273	48 126	0.9990	0.203	0.614	102
26	36.31	Rutin	Flavonol	204, 255, 354	360	0.83–16.50	−3310	12 905	0.9990	0.228	0.692	99
27	37.22	Ellagic acid	Hydroxybenzoic acid	254	254	0.86–17.10	−41 982	75 271	0.9988	0.276	0.836	86
28	40.81	Myricetin	Flavonol	207, 253, 370	360	0.77–15.30	−10 951	23 849	0.9983	0.272	0.823	87
29	42.47	*trans*-Resveratrol	Stilbene	216, 305	315	0.77–15.45	−17 009	69 118	0.9994	0.153	0.465	97
30	44.18	Cinnamic acid	Cinnamic acid	204, 216, 277	280	0.80–16.05	−8569	79 329	0.9999	0.071	0.216	98
31	44.77	Quercetin	Flavonol	203, 254, 370	360	0.75–15.00	−16 388	43 127	0.9996	0.121	0.368	96
32	48.44	Kaempferol	Flavonol	200, 265, 364	360	0.85–16.95	−21 875	47 608	0.9998	0.111	0.337	96

a) *y*-axis intercept.
b) Slope of the regression line.

mobile phase and requiring a maximum of 50% of organic solvent to elute the analytes under study, avoiding high consumption of the organic phase, which frequently represents a significant cost in laboratories. Figure 2 shows typical chromatograms obtained applying this gradient to a polyphenols and furans standard solution and a wine sample.

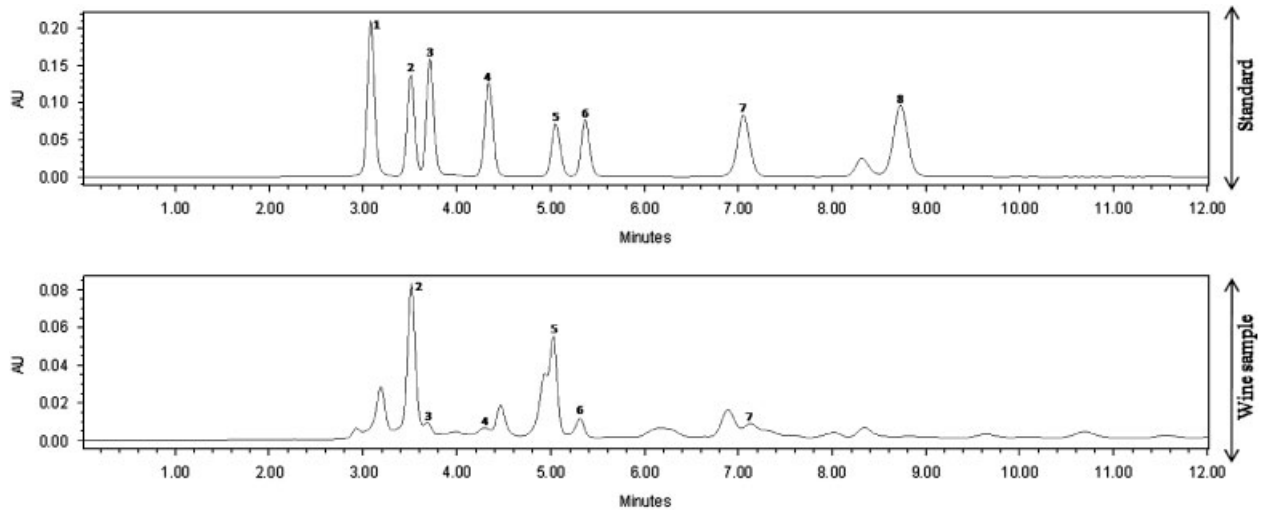

Figure 1. Representative chromatograms obtained with the proposed method for the determination of organic acids at 210 nm, when applied to the standard solution and a wine sample. See Table 2 for peak identification.

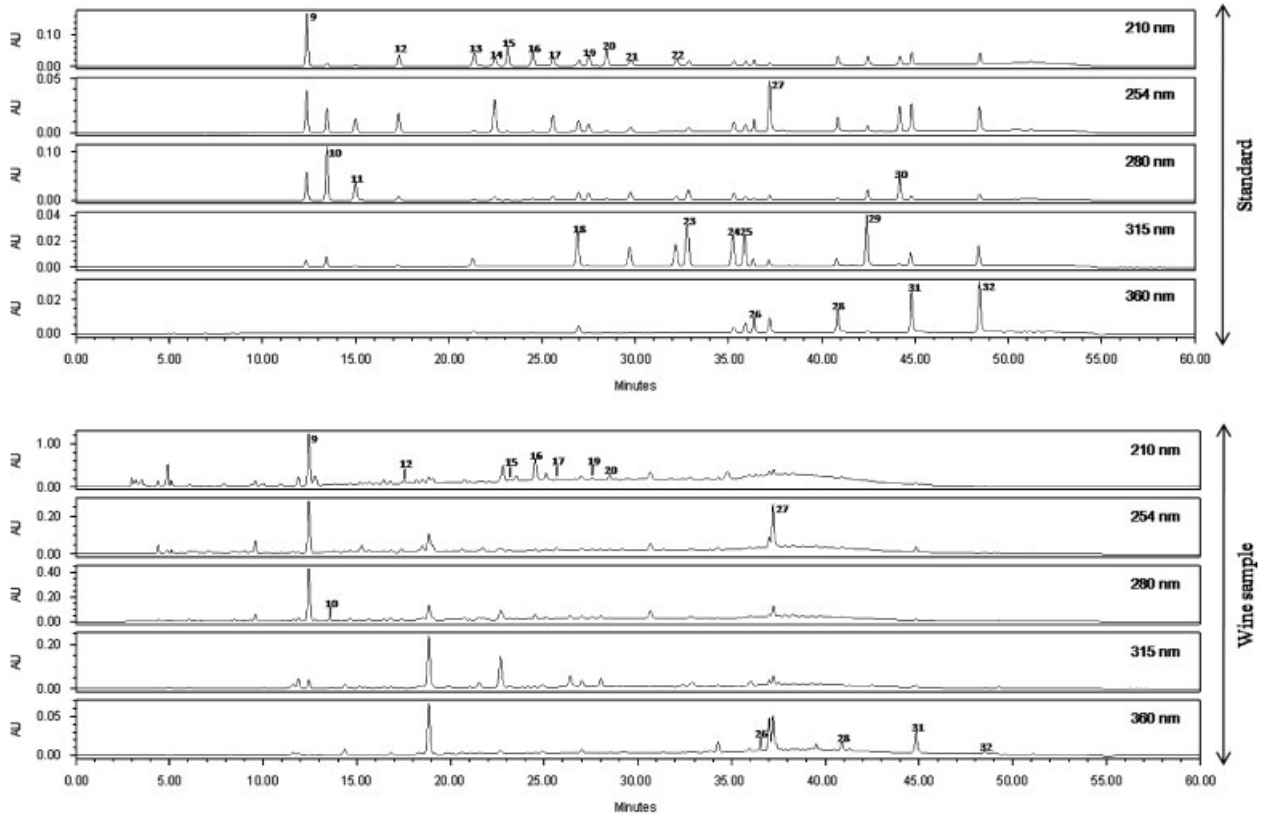

Figure 2. Representative chromatograms obtained with the proposed method for the determination of polyphenols and furans at the selected detection wavelengths: 210, 254, 280, 315 and 360 nm, when applied to a standard solution and a wine sample. See Table 2 for peak identification.

Phenolic acids are currently detected at 280 nm, even if most of them have higher absorption at wavelengths close to 210 nm, as flavan-3-ols. The spectral bands of the studied compounds were obtained by their spectral array between 190 and 600 nm and are summarized in Table 2. The detection wavelength was chosen near to the absorption maximum, except for the compounds which elute at the final stage of the analysis, as the influence

of the acetonitrile absorption increases at lower wavelengths. The use of different detection wavelengths ensured the compromise between selectivity and sensitivity. As published analytical methods usually require sample pretreatment and long time analysis, this study intended to overcome this, in order to obtain an easier methodology. Wine phenolic composition was then determined by direct injection of wine samples, after being filtered through 0.45 μm membrane filters. The direct injection of the samples was selected after testing other presample treatments, including SPE, without losing selectivity and resolution of the compounds of interest due to wine matrix (including the high alcohol content). Thus using the optimized conditions, well-resolved chromatograms of wine samples were obtained as shown in Fig. 2. This method also upgrades other previously proposed methods for the

Table 3. Repeatability (intra-day) and reproducibility (inter-day) of the developed method, expressed in terms of the variation (RSD%) of retention times (t_R) and areas

Compounds	S1	Intra-day[a)]		Inter-day[b)]		S2	Intra-day[a)]		Inter-day[b)]		S3	Intra-day[a)]		Inter-day[b)]	
		t_R RSD%	Area RSD%	t_R RSD%	Area RSD%		t_R RSD%	Area RSD%	t_R RSD%	Area RSD%		t_R RSD%	Area RSD%	t_R RSD%	Area RSD%
	g/L					g/L					g/L				
Oxalic acid	0.077	0.2	0.1	0.2	1.9	0.154	0.1	0.1	0.2	4.0	0.230	0.1	0.1	0.2	0.8
Tartaric acid	0.378	0.2	0.5	0.2	1.5	0.756	0.1	0.4	0.2	3.6	1.134	0.2	0.3	0.3	0.6
Formic acid	0.750	0.1	0.3	0.1	2.2	1.501	0.1	0.3	0.2	3.9	2.251	0.1	0.3	0.2	1.3
Malic acid	0.761	0.1	0.3	0.2	1.5	1.523	0.1	0.1	0.2	3.4	2.284	0.2	0.1	0.4	0.3
Lactic acid	1.494	0.1	0.5	0.2	5.7	2.988	0.1	0.7	0.1	9.0	4.482	0.1	1.0	0.3	5.9
Acetic acid	1.496	0.1	0.5	1.7	0.3	2.993	0.1	0.6	1.4	2.8	4.489	0.2	1.0	1.6	0.7
Citric acid	0.563	0.1	0.1	1.1	1.8	1.126	0.1	0.1	1.1	3.5	1.689	0.1	0.1	1.4	0.5
Succinic acid	0.386	0.0	0.1	0.4	1.6	0.771	0.1	0.1	0.5	3.5	1.157	0.2	0.1	0.8	0.6
	mg/L					mg/L					mg/L				
Gallic acid	5.40	0.0	0.3	0.1	0.1	18.90	0.3	0.3	1.2	0.4	40.50	0.0	0.2	1.2	0.1
HMF	3.00	0.0	0.2	0.1	0.1	10.50	0.2	0.2	0.8	0.2	22.50	0.1	0.1	0.8	0.3
Furfural	1.50	0.0	0.6	0.1	1.1	5.25	0.1	0.4	0.7	2.2	11.25	0.1	0.1	0.7	1.1
Protocatechuic acid	1.59	0.0	0.2	0.1	0.5	5.57	0.3	0.5	1.1	1.3	11.93	0.1	0.3	1.3	0.2
Gentisic acid	1.61	0.1	0.5	0.1	4.6	5.62	0.3	0.3	1.0	2.9	12.04	0.1	0.2	1.3	3.4
p-Hydroxybenzoic acid	1.50	0.0	1.2	0.1	4.0	5.25	0.2	0.8	0.9	3.7	11.25	0.1	0.2	1.1	3.4
(−)-Epigallocatechin	1.50	0.0	0.2	0.1	1.3	5.25	0.2	0.4	0.6	0.8	11.25	0.1	0.4	0.9	1.4
(+)-Catechin	1.50	0.0	0.4	0.2	2.3	5.25	0.2	0.3	0.7	0.3	11.25	0.1	0.1	1.0	0.1
Vanillic acid	1.58	0.0	0.6	0.0	1.4	5.51	0.2	0.4	0.6	0.4	11.81	0.1	0.2	0.8	0.5
Caffeic acid	1.68	0.0	0.5	0.2	1.3	5.88	0.2	0.3	0.7	0.3	12.60	0.1	0.2	0.9	0.5
Syringic acid	1.50	0.0	0.8	0.0	0.7	5.25	0.2	0.3	0.5	0.6	11.25	0.1	0.2	0.7	0.2
(−)-Epicatechin	1.61	0.0	0.2	0.1	0.9	5.62	0.2	0.3	0.6	1.1	12.04	0.1	0.2	0.8	0.5
Vanillin	1.50	0.0	1.2	0.0	1.2	5.25	0.2	0.3	0.6	1.6	11.25	0.1	0.1	0.8	1.0
Syringaldehyde	1.56	0.0	0.9	0.0	2.7	5.46	0.1	0.6	0.4	1.2	11.70	0.0	0.3	0.6	0.2
p-Coumaric acid	1.58	0.0	0.4	0.1	2.6	5.51	0.2	0.5	0.6	0.5	11.81	0.1	0.1	0.9	0.4
Ferulic acid	1.58	0.0	0.9	0.2	1.2	5.51	0.1	0.5	0.4	0.6	11.81	0.0	0.3	0.6	0.6
Sinapic acid	1.53	0.0	0.5	0.3	0.5	5.36	0.1	0.6	0.3	0.5	11.48	0.0	0.3	0.5	1.5
Rutin	1.65	0.0	2.9	0.4	7.4	5.78	0.1	0.5	0.2	1.3	12.38	0.0	0.9	0.3	1.4
Ellagic acid	1.71	0.0	1.1	0.2	7.2	5.99	0.1	0.8	0.2	4.5	12.83	0.0	1.0	0.4	2.0
Myricetin	1.53	0.0	3.1	0.3	6.0	5.36	0.1	1.3	0.3	3.1	11.48	0.0	0.6	0.3	1.8
trans-Resveratrol	1.55	0.0	0.7	0.1	2.3	5.41	0.1	0.4	0.3	0.8	11.59	0.0	0.1	0.4	0.1
Cinnamic acid	1.61	0.0	0.4	0.0	0.3	5.62	0.1	0.2	0.3	0.5	12.04	0.0	0.1	0.3	0.1
Quercetin	1.50	0.0	1.4	0.0	1.1	5.25	0.1	0.9	0.3	1.9	11.25	0.0	0.4	0.3	1.5
Kaempferol	1.70	0.0	1.4	0.3	4.8	5.93	0.1	0.9	0.3	1.4	12.71	0.0	0.4	0.3	1.2

a) $n = 5$.

b) Three different days $n = 15$; S1, S2 and S3 are standards at different concentrations; t_R – retention time.

Table 4. Concentrations found in the studied wine varieties by application of the developed method[a)]

Wines	F1		F2		F3		F4		R1		R2		R3		R4		W1		W2		W3		W4		Rs	
Compound	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%	Cc	RSD%
g/L																										
Oxalic acid	0.061	0.8	0.055	6.0	n.d.		0.066	3.7	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		0.043	1.0	0.031	0.6
Tartaric acid	1.726	0.1	1.613	0.3	2.193	0.1	2.734	0.4	1.704	0.5	0.545	0.3	2.422	0.7	1.682	2.0	1.274	1.0	1.508	0.4	1.328	0.8	1.681	0.4	0.725	2.4
Formic acid	0.246	3.6	0.449	1.5	0.372	1.5	0.363	2.4	0.346	5.4	0.452	3.8	0.404	0.6	0.431	1.9	0.514	3.4	0.508	3.3	0.440	4.2	0.595	2.9	0.417	3.5
Malic acid	3.551	0.5	3.506	2.3	2.652	1.1	0.765	6.1	0.351	5.4	0.373	3.6	0.252	4.7	0.249	1.2	3.118	2.7	1.859	3.6	2.256	1.9	2.845	1.2	3.642	0.2
Lactic acid	1.070	4.4	1.469	5.3	2.102	5.5	6.273	3.5	7.406	3.7	6.248	5.0	9.839	1.9	7.562	2.6	n.d.		1.331	6.6	1.175	6.3	1.424	3.3	0.821	6.0
Acetic acid	0.666	3.3	1.627	5.0	0.974	3.4	1.734	8.4	1.512	0.4	1.599	1.9	2.207	0.4	2.090	2.5	0.826	2.8	0.871	4.3	0.929	5.2	0.956	2.1	1.067	0.4
Citric acid	n.d.		n.d.		0.319	1.6	n.d.		0.421	2.7	n.d.		n.d.		0.509	3.3	0.236	0.7	0.291	0.5	0.333	0.4	0.362	1.3	0.300	2.3
Succinic acid	n.d.		n.d.		n.d.		n.d.		n.d.		0.063	1.2	n.d.		0.071	3.8	0.124	2.1	0.152	1.8	0.109	1.2	0.131	4.0	0.137	0.6
mg /L																										
Gallic acid	5.18	0.5	6.13	0.6	5.82	0.1	5.96	0.3	31.94	0.2	24.07	0.5	17.57	4.8	37.26	1.5	7.42	0.3	6.51	0.2	1.84	0.6	2.59	4.3	11.64	0.2
HMF	15.47	0.2	338.76	0.1	8.71	0.5	118.96	0.4	1.11	11.4	n.q.		n.q.		n.q.		0.84	0.2	n.q.		1.76	0.3	n.q.		n.q.	
Furfural	1.61	0.5	10.40	0.4	2.01	0.4	8.75	0.5	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Protocatechuic acid	2.67	2.5	3.46	0.2	3.80	0.8	4.66	2.9	2.95	7.9	2.89	7.4	n.d.		3.32	2.7	2.67	1.1	2.78	0.4	2.25	0.4	1.83	0.2	2.84	4.3
Gentisic acid	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		1.45	5.2	1.29	0.6	n.d.		1.26	0.5	n.d.	
p-Hydroxybenzoic acid	n.d.		1.61	0.6	1.83	2.2	3.36	2.2	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
(−)-Epigallocatechin	1.05	1.5	1.41	0.1	1.31	2.6	1.89	2.1	4.53	7.6	3.42	8.0	7.76	1.8	3.45	3.7	1.51	0.9	n.q.		0.95	1.2	n.q.		1.95	0.5
(+)-Catechin	n.d.		n.d.		0.90	5.8	0.76	7.9	13.68	2.0	13.59	0.3	17.32	0.3	8.88	2.8	5.18	2.1	16.12	0.1	7.40	0.4	7.47	0.1	8.22	1.3
Vanillic acid	n.d.		0.76	0.7	0.76	4.9	n.d.		5.40	4.1	6.23	0.6	5.16	0.5	8.92	2.7	0.75	1.1	n.d.		1.37	2.2	n.d.		1.71	1.1
Caffeic acid	1.95	0.5	1.34	0.2	2.53	2.3	0.76	3.2	3.34	1.0	1.41	2.8	3.03	0.5	4.00	0.2	4.36	0.6	2.48	0.2	5.62	0.2	2.38	0.2	3.15	0.2
Syringic acid	4.62	0.8	0.70	1.8	0.53	2.6	1.00	1.2	6.14	3.8	5.35	1.3	1.34	1.9	7.59	5.0	n.d.		n.d.		n.d.		n.d.		1.33	2.0
(−)-Epicatechin	n.d.		n.d.		n.d.		n.d.		3.57	8.6	7.33	3.2	7.63	1.9	3.15	1.1	1.47	1.3	10.16	0.4	2.97	3.7	3.73	0.3	3.03	0.6
Vanillin	n.d.		0.72	1.7	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Syringaldehyde	1.69	1.0	2.95	1.0	2.49	4.4	1.91	2.9	n.d.		n.d.		n.d.		2.42	2.4	n.d.		n.d.		n.d.		n.d.		n.d.	
p-Coumaric acid	1.23	0.5	1.64	4.3	1.29	3.3	1.04	3.2	2.59	2.2	0.89	1.7	1.83	1.4	1.22	3.2	1.90	0.2	2.05	0.1	2.27	0.3	2.24	0.2	2.08	0.5
Ferulic acid	n.q.		n.q.		n.q.		n.q.		n.q.		n.d.		0.61	6.7	n.d.		0.64	0.7	n.q.		0.86	0.5	n.q.		n.q.	
Sinapic acid	1.76	2.2	1.16	2.8	2.27	0.4	1.32	1.5	2.79	3.0	3.09	4.6	4.86	7.6	n.d.		1.44	0.6	n.q.		0.61	1.5	0.64	0.7	4.08	0.8
Rutin	n.d.		n.d.		n.d.		n.d.		1.86	10.5	n.d.		n.d.		n.q.		n.d.		n.d.		n.d.		n.d.		n.d.	
Ellagic acid	3.05	2.7	2.33	0.7	2.42	1.1	3.52	1.0	14.67	1.2	4.64	0.1	5.28	0.6	7.94	0.8	n.q.		n.d.		n.q.		n.d.		n.d.	
Myricetin	n.d.		n.d.		n.d.		n.d.		2.46	5.2	0.82	4.7	1.71	3.1	1.48	4.5	n.q.		n.d.		n.d.		n.d.		n.q.	
trans-Resveratrol	n.d.		n.d.		n.d.		n.d.		0.70	5.3	1.28	0.4	1.05	7.1	0.84	0.9	n.q.		0.49	1.1	n.q.		n.q.		0.82	1.1
Cinnamic acid	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Quercetin	n.d.		n.d.		n.d.		n.d.		3.37	0.4	1.29	2.4	3.61	1.1	2.58	0.2	n.d.		n.d.		0.44	2.1	0.43	2.0	0.38	3.8
Kaempferol	n.d.		n.d.		n.d.		n.d.		0.72	1.0	n.q.		0.69	2.0	0.46	1.3	n.d.		n.d.		n.d.		n.d.		n.d.	

[a)]Cc – concentration; n.d. – not detected, lower than LOD; n.q. – not quantified, lower than LOQ.

simultaneous analysis of organic acids and polyphenols [11], maintaining the basic principles but improving sensitivity and chromatographic resolution as well as the number of target compounds (up to 32).

3.2 Validation procedure

In order to validate the developed methodology, several parameters such as linearity, analytical determination limits, recovery, precision and accuracy were considered.

The linearity was evaluated by the analysis in triplicate of six standards solutions. The obtained validation parameters are listed in Table 2. Good correlation coefficients (R^2) were observed, higher than 0.9982 for polyphenols and furans and 0.9997 for organic acids, confirming the linearity of the method.

Method sensitivity was evaluated by LOD and LOQ determinations, calculated on the basis of the linear regression curves. The LODs were in the range of 0.07–0.49 mg/L for polyphenols (cinnamic and gallic acids) and furans and 0.001–0.046 g/L for the organic acids (oxalic and lactic acids). Given that the LODs and LOQs are considerably low (Table 2), it is reasonable to conclude that this method can be used for quantitative analysis in wines. The LODs results are comparable or lower than those found in the literature [49, 53–55].

Recovery studies were carried out to determine the accuracy of the method. A wine sample was analyzed before and after the addition of different known amounts of organic acids, furans and polyphenols, and recoveries ranged between 80 and 104% for furans and polyphenols and 97–105% for organic acids were found. These results reveal that the matrix composition complexity does not compromise selectivity and sensitivity of the method, allowing the direct analysis of wines.

The method precision (repeatability and reproducibility) was evaluated by the assessment of five successive analyses of standard working solutions, at three different concentrations, by intra- and inter-day (three different days) repetition method. The precision is expressed in terms of the variation (RSD%) of retention times (t_R) and areas obtained for the repeatability and reproducibility tests (Table 3). The small variation of t_R (with a maximum of 1.7%) is very important in order to avoid misidentification of peaks in wine samples (Fig. 2). The area variation is, in general, small but higher for the reproducibility tests, with maxima at 7.4% for phenolics and 9.0% for organic acids as summarized in Table 3.

3.3 Wine sample analysis

In order to test the developed methodology in red, white, rosé and fortified wines, the samples were simply filtered (0.45 μm) and diluted, when necessary, to apply to the constructed calibration curves. For the purpose of this study, quantified results slightly below the previous validated working range were confirmed by increasing the injection volume. The obtained results are summarized in Table 4.

Regarding the organic acid analysis, the attained results vary from 0.055 to 6.273 g/L in fortified wines for oxalic and lactic acids, 0.063 to 9.839 g/L in red wines for succinic and lactic acids, 0.043 to 3.118 g/L in white wines and 0.031 to 3.642 g/L in rosé wine, for oxalic and malic acids, respectively. As can be shown, the concentration of organic acids found in wines varies significantly between wine type and also from one sample to another, suggesting that it is strongly dependent on wine nature and therefore on the vinification process applied. Cunha *et al.* [9] and Esteves *et al.* [68] also report variable concentrations when they analyzed tartaric, malic, lactic, succinic and acetic acids in fortified wines, with values between 0.219 and 1.442 g/L and between 0.041 and 2.752 g/L, respectively. The same result was obtained by Villiers *et al.* [69] when determining the same compounds in red and white wines.

Polyphenols in fortified wines ranged between 0.53 and 6.13 mg/L, between 0.46 and 37.26 mg/L in red wines, between 0.43 and 16.12 mg/L in white wines and between 0.38 and 11.64 mg/L in the rosé wine. These values are in the range of the amounts found in other red [40, 46, 51, 54], white [46, 70] and fortified [71] wine varieties, showing that the results obtained in this study are acceptable and coherent. In addition, furans were also determined as they are present mainly in fortified wines, showing maximum results of 338.76 and 10.40 mg/L for HMF and furfural, respectively. As similar results were obtained by Ho *et al.* [71], the above application demonstrates the effectiveness of the developed method for the determination of these compounds in fortified wines.

4 Concluding remarks

A simple and rapid method was developed for the sequential determination of organic acids, furans and phenolic compounds in different wine matrices by HPLC technology. This method combines sensitivity with time-effectiveness and was successfully used to measure and assess the polyphenolic fingerprint and organic acids profile of red, white, rosé and fortified wines. The determination of two furanic compounds, HMF and furfural, frequently detected in fortified wines, was also performed by the present method. Furthermore, the methodology provides the potential to analyze wine samples in a single chromatographic column and avoiding tedious and time consuming sample preparation procedures. Therefore, 22 of the most common phenolic compounds and furans in wines were separated in 60 min and eight organic acids in 12 min, allowing simultaneous quality control analysis. The methodology can be extended to the determination of other wine polyphenols if additional calibrating standards are used.

This work was supported in part by project ANTIVINMAC (05/MAC/2.3/M28) from the program INTERREG-IIIB/FEDER. Vanda Pereira was supported by Fundação para a Ciência e Tecnologia, Ph.D. grant SFRH/BD/24177/2005.

The authors declared no conflict of interest.

5 References

[1] Ibern-Gomez, M., Andres-Lacueva, C., Lamuela-Raventos, R. M., Waterhouse, A. L., *Am. J. Enol. Viticult.* 2002, *53*, 218–221.

[2] Romero, E. G., Munoz, G. S., *J. Chromatogr. A* 1993, *655*, 111–117.

[3] Lamikanra, O., Inyang, I., Leong, S., *J. Agric. Food Chem.* 1995, *43*, 3026–3028.

[4] Goldberg, D. M., Yan, J., Ng, E., Diamandis, E. P., Karumanchiri, A., Soleas, G., Waterhouse, A. L., *Am. J. Enol. Viticult.* 1995, *46*, 159–165.

[5] McDonald, M. S., Hughes, M., Burns, J., Lean, M. E. J., Matthews, D., Crozier, A., *J. Agric. Food Chem.* 1998, *46*, 368–375.

[6] Pérez-Magariño, S., José, M. L. G.-S., *J. Agric. Food Chem.* 2004, *52*, 1181–1189.

[7] Belitz, H. D., Grosch, W., *Food Chemistry*, Springer, New York 1999.

[8] Mato, I., Suárez-Luque, S., Huidobro, J. F., *Food Res. Int.* 2005, *38*, 1175–1188.

[9] Cunha, S. C., Fernandes, J. O., Faria, M. A., Ferreira, I. M. P. L. V. O., Ferreira, M. A., *Cienc. Tecnol. Aliment.* 2002, *3*, 212–216.

[10] Soyer, Y., Koca, N., Karadeniz, F., *J. Food Compos. Anal.* 2003, *16*, 629–636.

[11] Kerem, Z., Bravdo, B.-a., Shoseyov, O., Tugendhaft, Y., *J. Chromatogr. A* 2004, *1052*, 211–215.

[12] Valentão, P., Seabra, R. M., Lopes, G., Silva, L. R., Martins, V., Trujillo, M. E., Velázquez, E., Andrade, P. B., *Food Chem.* 2007,*100*, 64–70.

[13] Cabrita, M. J., Torres, M., Palma, V., Alves, E., Patão, R., Costa Freitas, A. M., *Talanta* 2008, *74*, 1281–1286.

[14] López-Tamames, E., Puig-Deu, M. A., Teixeira, E., Buxaderas, S., *Am. J. Enol. Viticult.* 1996, *47*, 193–198.

[15] Falqué-López, E., Fernández-Gómez, E., *J. Chromatogr. Sci.* 1996, *34*, 254–257.

[16] Llorente, M., Villarroya, B., Gómez-Cordovés, C., *Chromatographia* 1991,*32*, 555–558.

[17] Pazo, M., Traveso, C., Cisneros, M. C., Montero, E., *Alimentaria* 1999, *36*, 139–142.

[18] Samanidou, V. F., Antoniou, C. V., Papadoyannis, I. N., *J. Liq. Chromatogr. Relat. Technol.* 2001, *24*, 2161–2176.

[19] Dong, M. W., *LC GC* 1998, *16*, 1092–1097.

[20] Walker, T., Morris, J., Threlfall, R., Main, G., *J. Agric. Food Chem.* 2003, *51*, 1543–1547.

[21] Robichaud, J. L., Noble, A. C., *J. Sci. Food Agric.* 1990, *53*, 343–353.

[22] Cheynier, V., Silva, J. M. R. d., *J. Agr. Food Chem.* 1991, *39*, 1047–1049.

[23] Pérez-Lamela, C., García-Falcón, M. S., Simal-Gándara, J., Orriols-Fernández, I., *Food Chem.* 2007, *101*, 601–606.

[24] Lairon, D., Amiot, M., *Curr. Opin. Lipidol.* 1999, *10*, 23–28.

[25] Morton, L., Caccetta, R. A.-A., Puddey, I., Croft, K., *Clin. Exp. Pharmacol. Physiol.* 2000, *27*, 152–159.

[26] Lule, S., Xia, W., *Food Rev. Int.* 2005, *21*, 367–388.

[27] Dell'Agli, M., Buscialá, A., Bosssio, E., *Cardiovasc. Res.* 2004, *63*, 593–602.

[28] Bravo, M. N., Silva, S., Coelho, A. V., Boas, L. V., Bronze, M. R., *Anal. Chim. Acta* 2006, *563*, 84–92.

[29] Fernández-Pachón, M. S., Villaño, D., Troncoso, A. M., García-Parrilla, M. C., *Anal. Chim. Acta* 2006, *563*, 101–108.

[30] Kallithraka, S., Tsoutsouras, E., Tzourou, E., Lanaridis, P., *Food Chem.* 2006, *99*, 784–793.

[31] Goldberg, D. M., Yan, J., Ng, E., Diamandls, E. P., Karumanchir, A., Soleas, G., Waterhouse, A. L., *Anal. Chem.* 1994, *66*, 3959–3963.

[32] Soleas, G. J., Dam, J., Carey, M., Goldberg, D. M., *J. Agric. Food Chem.* 1997, *45*, 3871–3880.

[33] Luan, T., Li, G., Zhang, Z., *Anal. Chim. Acta* 2000, *424*, 19–25.

[34] Minuti, L., Pellegrino, R. M., Tesei, I., *J. Chromatogr. A* 2006, *1114*, 263–268.

[35] Pazourek, J., Gajdošová, D., Spanilá, M., Farková, M., Novotná, K., Havel, J., *J. Chromatogr. A* 2005, *1081*, 48–54.

[36] Gu, X., Chu, Q., O'Dwyer, M., Zeece, M., *J. Chromatogr. A* 2000, *881*, 471–481.

[37] Capote, F. P., Rodríguez, J. M. L., Castro, M. D. L. d., *J. Chromatogr. A* 2007, *1139*, 301–307.

[38] García-Falcóna, M. S., Pérez-Lamela, C., Martínez-Carballo, E., Simal-Gándara, J., *Food Chem.* 2007, *105*, 248–259.

[39] Makris, D. P., Kallithraka, S., Mamalos, A., *Talanta* 2006, *70*, 1143–1152.

[40] Silva, L. R., Andrade, P. B., Valentão, P., Seabra, R. M., Trujillo, M. E., Velázquez, E., *Food Chem.* 2005, *89*, 185–189.

[41] Nave, F., Cabrita, M. J., Costa, C. T. d., *J. Chromatogr. A* 2007, *1169*, 23–30.

[42] Abad-García, B., Berrueta, L. A., López-Márquez, D. M., Crespo-Ferrer, I., Gallo, B., Vicente, F., *J. Chromatogr. A* 2007, *1154*, 87–96.

[43] Vitrac, X., Monti, J.-P., Vercauteren, J., Deffieux, G., Mérillon, J.-M., *Anal. Chim. Acta* 2002, *458*, 103–110.

[44] Garcia-Viguera, C., Br idle, P., *Food Chem.* 1995, *54*, 349–352.

[45] Arnous, A., Makris, D. P., Kefalas, P., *J. Agric. Food Chem.* 2001, *49*, 5736–5742.

[46] Castellari, M., Sartini, E., Fabiani, A., Arfelli, G., Amati, A., *J. Chromatogr. A* 2002, *973*, 221–227.

[47] Canas, S., Belchior, A. P., Spranger, M. I., Bruno-de-Sousa, R., *J. Sep. Sci.* 2003, *26*, 496–502.

[48] Robbins, R. J., Bean, S. R., *J. Chromatogr. A* 2004, *1038*, 97–105.

[49] Sanza, M. A., Domínguez, I. N., Cárcel Cárcel, L. M., Gracia, L. N., *Anal. Chim. Acta* 2004, *513*, 229–237.

[50] Gutiérrez, I. H., Lorenzo, E. S., Espinosa, A. V., *Food Chem.* 2005, *92*, 269–283.

[51] Monagas, M., Suárez, R., Gómez-Cordovés, C., Bartolomé, B., *Am. J. Enol. Viticult.* 2005, *56*, 139–147.

[52] Recamales, A. F., Sayago, A., González-Miret, M. L., Hernanz, D., *Food Res. Int.* 2006, *39*, 220–229.

[53] Gomez-Alonso, S., Garcia-Romero, E., Hermosin-Gutierrez, I., *J. Food Compos. Anal.* 2007, *20*, 618–626.

[54] Tarola, A. M., Giannetti, V., *Chromatographia* 2007, *65*, 367–371.

[55] Paixão, N., Pereira, V., Marques, J. C., Câmara, J. S., *J. Sep. Sci.* 2008, *31*, 2189–2198.

[56] Preys, S., Mazerolles, G., Courcoux, P., Samson, A., Fischer, U., Hanafi, M., Bertrand, D., Cheynier, V., *Anal. Chim. Acta* 2006, *563*, 126–136.

[57] Bravo, M. N., Feliciano, R., Silva, S., Coelho, A. V., Boas, L. V., Bronze, M. R., *J. Food Compos. Anal.* 2008, *21*, 634–643.

[58] El Kaoutit, M., Naranjo-Rodriguez, I., Temsamani, K. R., Hernández-Artiga, M. P., Bellido-Milla, D., Cisneros, J. L. H.-H. d., *Food Chem.* 2008, *110*, 1019–1024.

[59] Borbalán, Á. M. A., Zorro, L., Guillén, D. A., Barroso, C. G., *J. Chromatogr. A* 2003, *1012*, 31–38.

[60] Pérez-Magariño, S., Revilla, I., González-San José, M. L., Beltrán, S., *J. Chromatogr. A* 1999, *847*, 75–81.

[61] Bilbao, M. d. L. M., Andrés-Lacueva, C., Jáuregui, O., Lamuela-Raventós, R. M., *Food Chem.* 2007, *101*, 1742–1747.

[62] Seeram, N. P., Lee, R., Scheuller, H. S., Heber, D., *Food Chem.* 2006, *97*, 1–11.

[63] Griffith, A. P., Collison, M. W., *J. Chromatogr. A* 2001, *913*, 397–413.

[64] Kao, T. H., Huang, S. C., Inbaraj, B. S., Chen, B. H., *Anal. Chim. Acta* 2008, *626*, 200–211.

[65] Ding, M. Y., Koizumi, H., Suzuki, Y., *Anal. Sci.* 1995, *11*, 239–243.

[66] Dopico-García,M. S., Valentão, P., Guerra, L., Andrade, P. B., Seabra, R. M., *Anal. Chim. Acta* 2007, *583*, 15–22.

[67] Waters, Atlantis Columns Applications Notebook, 2004.

[68] Esteves, V. I., Lima, S. S. F., Lima, D. L. D., Duarte, A. C., *Anal. Chim. Acta* 2004, *513*, 163–167.

[69] Villiers, A., Lynen, F., Crouch, A., Sandra, P., *Eur. Food Res. Technol.* 2003, *217*, 535–540.

[70] Darias-Martín, J. J. , Andrés-Lacueva, C., Díaz-Romero, C., Lamuela-Raventós, R. M., *Eur. Food Res. Technol.* 2008, *226*, 871–876.

[71] Ho, P., Hogg, T. A., Silva, M. C. M., *Food Chem.* 1999, *64*, 115–122.

