ELSEVIER

Journal of Chromatography A, 912 (2001) 249–257

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection ☆

M.A. Rodríguez-Delgado[a,*], S. Malovaná[b], J.P. Pérez[a], T. Borges[a], F.J. García Montelongo[a]

[a]*Department of Analytical Chemistry, Nutrition and Food Science, University of La Laguna, 38071, Tenerife, Spain*
[b]*Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlárská 2 CZ-61137 Brno, Czech Republic*

Received 20 November 2000; received in revised form 17 January 2001; accepted 25 January 2001

Abstract

Phenolic compounds including phenolic aldehydes, acids and flavonoids are separated by high-performance liquid chromatography (HPLC) with analysis time shorter than described in the literature. The use of a fluorescence detector in series with absorbance detector allowed increasing selectivity and sensitivity for the determination of catechin, vanillic acid, syringic acid, epicatechin and *trans*-resveratrol in wine samples. An optimised sample preparation method using liquid–liquid extraction with diethyl ether at pH 2.0 was used. The optimised method was applied to analyse wine samples with good results. © 2001 Published by Elsevier Science B.V.

Keywords: Wine; Food analysis; Phenolic compounds

1. Introduction

Phenolic compounds are an important group of substances that contribute to several sensorial characteristics such as colour, flavour, astringency and hardness of wine. Furthermore these compounds are important in food hygiene due to their bactericidal effects and consequently they are essential in the quality of a wine [1–4]. Some of them like *trans*-resveratrol have been identified as active ingredients, which contribute to the prevention of cancer, inhibiting tumour initiation, promotion and progression, and heart disease [5–7]. The types and concentrations of the phenolic compounds in wine depend on grape variety and ripening, atmospheric conditions and the techniques employed in producing the must, and on aging. It has been demonstrated that *trans*-resveratrol content is marked by temperature, thus its concentration is lower when warmer and drier conditions prevail along the manufacturing process [8].

Different techniques have been used for the determination of these compounds in wine samples, with thin-layer chromatographic (TLC) and high-performance liquid chromatographic (HPLC) [9–14] being the most used. More recently capillary electrophoresis (CE) has been shown to be a fast, powerful, clean and efficient separation technique for a wide variety of these compounds [15–19]. Although

☆Presented at the 29th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Alcala de Henares (Madrid), 12–14 July 2000.

*Corresponding author. Tel.: +34-922-318-046; fax: +34-922-318-003.

E-mail address: mrguez@ull.es (M.A. Rodríguez-Delgado).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00598-2

HPLC or CE methods use absorbance or photodiode-array detectors, which require sometimes the application of chemometric tools to increase the selectivity of the determination, the use of a programmable excitation and emission wavelengths spectrofluorimetric detector decreases the threshold of measurable concentration and increases the selectivity.

The difficulties in interpreting different chromatograms make necessary the optimisation of sample preparation in function of the polyphenolic compounds that have to be analysed. From the bibliography it can be concluded that there is controversy in sample preparation methods, some authors using solid-phase extraction with C_{18} or strong anion-exchange (SAX) anionic cartridges, others using liquid–liquid extraction with different organic solvents like ethyl acetate or diethyl ether, and some others inject samples directly without any preparation step [20–26]. Depending on the nature of compounds recoveries can be very different, so it is possible to have a mixture with polar and non-polar compounds that requires an optimisation procedure to obtain the best results. In this sense an optimised extraction method has developed to determinate polyphenolic compounds in wine samples [27].

The aim of this investigation was developing a method to increase the selectivity and sensitivity in the determination of a group of phenolic compounds using a programmable fluorescence detector in series with an absorbance detector. Using the optimised method examples of analyses of phenolic compounds in red and white wine samples were carried out showing the advantages of use a fluorescence detector.

2. Experimental

2.1. Apparatus

The analyses were carried out in a Waters liquid chromatograph equipped with two pumps (Models 510), an automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20-μl loop), a programmable fluorescence detector (Model 470) and a tunable absorbance detector (Model 486). A Baseline Workstation 810 software (Waters) and a personal computer were employed for data storage and evaluation. The analytical column was a Waters (Mildford, MA, USA) Nova-Pak C_{18} 150×3.9-mm I.D., 4-μm particle diameter. A Nova-Pak C_{18} pre-column was employed to protect the analytical column.

To check the peak purity of each compound a Beckman diode array detector (Model 168) controlled by the Beckman System Gold software was used. Peaks were considered pure when there was a match factor ≥99.5.

2.2. Reagents

All chemicals were of analytical grade. Gallic acid (1), protocatechuic acid (2), protocatechuicaldehyde (3), catechin (4), 2,5-dihydroxybenzaldehyde (5), vanillic acid (6), caffeic acid (7), syringic acid (8), epicatechin (9), syringaldehyde (10), *p*-coumaric acid (11), ferulic acid (12), *trans*-resveratrol (13), myricetin (14), quercitrin (15), quercetin (16) and kaempferol (17) were obtained for Sigma (St. Louis, MO, USA). Standards were dissolved in a matrix solution (ethanol–3 g/l tartaric acid in water, 15:85, v/v) with concentrations in the range 1.8–3.6 mg/l and stored at −4°C in the darkness. Oxygen was eliminated from all solutions with a nitrogen stream to avoid decomposition of these compounds. Working standard solutions were prepared by diluting the stock solutions with the matrix solution. The polyphenolic compound 2,5-dihydroxybenzaldehyde was used as internal standard at concentration 34.4 mg/l. HPLC-grade methanol, acetic acid, diethyl ether and ethanol were obtained from Merck (Darmstadt, Germany). Hydrochloric acid was obtained from Merck. Ultrapure water from Milli-Q system (Millipore, Bedford, MA, USA) with a conductivity of 18 MΩ was used in all cases. All solution were filtered through 0.45-μm membranes (Millipore) and degassed prior to use.

2.3. Sample preparation

Samples of commercially available wines from the Canary Islands were analysed with the proposed method. The pH of wine samples was adjusted to pH 2 by adding small amounts of 0.1 *M* hydrochloric acid. Then, 5 ml of wine were extracted twice with 5 ml of diethyl ether for 20 min using a Selecta Rotabit

(Selecta, Barcelona, Spain) at 180 rpm. Organic layers were separated and evaporated to dryness with nitrogen gas stream. The dry residue was dissolved in methanol–water (1:1) and aliquots injected into the HPLC system. All samples were filtered through a Swinny Stainless of 13 mm equipped with cellulose acetate (Millipore) 0.45-μm filter. Duplicate injections were performed and average peak areas were used for the quantitation.

2.4. Chromatographic conditions

The chromatographic separation was carried out using as mobile phase methanol–acetic acid–water (10:2:88, v/v) as solvent A and methanol–acetic acid–water (90:2:8, v/v) as solvent B with the following gradient program:

Time (min)	A (%)	B (%)	Curve no.
0	100	0	–*
15	85	15	6
25	50	50	6
34	30	70	6

with a total flow-rate set of 1.0 ml/min. A wavelength of 280 nm was used for absorbance detector, while λ_{ex}=278 nm and λ_{em}=360 nm over 17.5 min and λ_{ex}=330 nm and λ_{em}=374 nm for 16.5 min. The bandwidth was ±2 nm for both detectors and a wavelength repeatability of ±0.25 nm for absorbance detector and ±0.30 nm for fluorescence detector. Injected samples were interspersed with standards to ensure accurate quantitation. Chromatographic peaks were identified by comparing retention times of samples with those of standard compounds. Quantitation was carried out by internal standardization.

3. Results and discussion

Pezet et al. [28] described the spectral properties of *trans*-resveratrol showing that this compound as well as another stilbenes show bright fluorescence at 366 nm when irradiated with ultraviolet light. Many phenolic compounds show maximum absorbance between 265 and 335 nm, while excitation and emission spectral are specific. Excitation and emission spectra for the polyphenolic compounds under investigation were obtained using the Waters 470 programmable wavelength fluorimetric detector in order to determinate the optimal excitation and emission wavelengths to carried out the detection in the chromatographic separation. Table 1 shows maximum excitation and emission wavelengths for each pure polyphenol dissolved in a solution of 3 g/l tartaric acid in water with 15% (v/v) of ethanol. Although it is possible determinate excitation and emission wavelengths for every compound, the use of these wavelengths is determined by their response in this fluorescence detector.

Excitation and emission wavelengths for the detection were automatically switched according several criteria: the elution characteristics of each compound, the previous knowledge of the phenolic compounds most found in samples of Canary Island wines and the ease with which the excitation and emission wavelengths can be precisely changed within the elution profile of the samples. Although there are several gradients described in the bibliography [12–14], the introduction of another important polyphenolic species like *trans*-resveratrol and the specific complexity of wine samples make necessary

Table 1
Excitation and emission wavelengths of polyphenolic compound

No.	Compound	Excitation (nm)	Emission (nm)
1	Gallic acid	278	366
2	Protocatechuic acid	270	358
3	Protocatechuicaldehyde	265	360
4	(+)-Catechine	278	360
5	2,5-Dihydroxybenzaldehyde	278	360
6	Vanillic acid	278	360
7	Caffeic acid	262	426
8	Syringic acid	278	360
9	(−)-Epicatechin	278	360
10	Syringaldehyde	260	422
11	*p*-Coumaric acid	260	422
12	Ferulic acid	260	422
13	*trans*-Resveratrol	330	374
14	Myricetin	268	370
15	Quercitrin	260	426
16	Quercetin	264	420
17	Kaempferol	268	422

an optimisation process to separate in the same run typical and new polyphenolic compounds whose content it is interesting to know to have beneficial effects to our health. With these parameters in mind several test were carried out in order to optimise the chromatographic elution gradient, which it should include the internal standard. Fig. 1 shows the separation of this group of compounds using the elution gradient described in Section 2 with the absorbance and fluorescence detectors in series which reduces analysis time from 45 to 35 min with respect to data in the bibliography. Besides, sensitivity for the determination of catechin, vanillic acid, syringic acid, epicatechin and *trans*-resveratrol increase considerably at concentrations near to those present in wine samples. Thus sensitivity increases

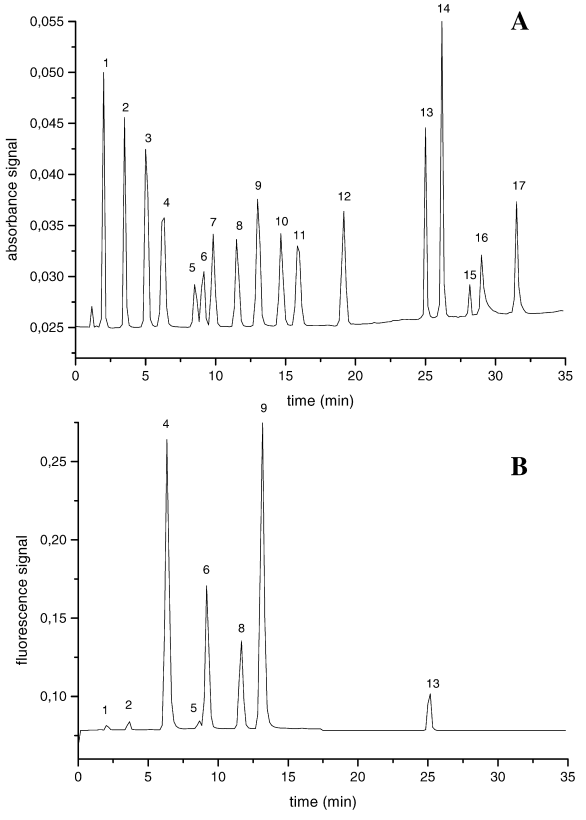

Fig. 1. Chromatograms of a standard mixture of polyphenolic compounds using: (A) absorbance detection (λ=280 nm), (B) fluorescence detection (λ_{ex}=278 nm and λ_{em}=360 nm over 17.5 min and λ_{ex}=330 nm and λ_{em}=374 nm for 16.5 min). Chromatographic conditions and identification are described in Section 2.

14 times for catechin, 16.5 times for vanillic acid, 7 times for syringic acid, 16.5 times for epicatechin and twice for *trans*-resveratrol. Fig. 1B shows that compounds like gallic acid and protocatechuic acid appear in both chromatograms but the sensitivity in the fluorescence mode is lower with regard to the response obtained with the absorbance detector. However, although the sensitivity is lower, the selectivity of the determination increases and allows discriminate between different compounds present in samples. Recently, Viñas et al. [29] have demonstrated the utility of a fluorescence detector coupled to a photodiode-array detection (DAD) system to analyse a reduced group of these compounds in wine samples. However from the profile chromatographic shown in the figures of Viñas et al.'s paper, it can be deduced that it is necessary a prior stage of sample preparation to obtain peaks with high efficiencies allowing an easier quantitation. In our paper, it has been demonstrated that with a simple sample preparation, efficiencies of peaks are higher and using fluorescence detector another compounds can be identified and quantified.

Table 2 shows reproducibility and repeatability values for retention time and peak area obtained with both detectors. It can be seen that reproducibility and repeatability for retention times is very high, with standard deviation lower than 2%, while these statistical parameters for peak area values are lower for all compounds except for the most hydrophobic quercetin and kaempferol, which can be justified because of the efficiency of these peaks is not very high. It can be seen too that standard deviations using fluorescence detection are lower than with the absorbance detector, which it allows a more accurate quantitation when using the fluorescence detector. Besides the values of retention time and areas are the same after 200 injections showing usefulness of the developed method for routine analysis.

The calibration graphs were obtained by injecting standard solutions in the range 0.02–40 mg/l (Table 3). Each point of calibration graph is the mean value from two independent area measurements. The precision between-day and within-day of the method was determined from replicate assays of wine spiked with known concentrations of polyphenolic compounds with RSD lower than 1.7%, except for quercetin and kaempferol for UV detection. For fluorescence detec-

Table 2
Repeatability and reproducibility for UV–Vis and fluorescence detection

No.	Compound	Conc. (mg/l)	Repeatability					Reproducibility			
			Time			Area		Time		Area	
			min	SD	RSD%	SD ($\times 10^5$)	RSD%	SD	RSD%	SD ($\times 10^5$)	RSD%
1	Gallic acid	3.6	1.98	0.016	0.79	0.019	0.81	0.013	0.64	0.019	0.79
2	Protocatechuic acid	5.2	3.52	0.043	1.23	0.020	0.98	0.029	0.83	0.017	0.86
3	Protocatechuicaldehyde	3.2	5.11	0.069	1.35	0.033	1.02	0.051	0.99	0.033	1.01
4	(+)-Catechine	16.8	6.28	0.097	1.55	0.023(0.281)[a]	0.94(0.79)[a]	0.079	1.26	0.022(0.195)[a]	0.91(0.55)[a]
5	2,5-Dihydroxybenzaldehyde	17.2	8.64	0.115	1.33	0.008	1.03	0.097	1.13	0.008	1.09
6	Vanillic acid	2.4	9.21	0.132	1.43	0.015(0.237)[a]	1.48(1.47)[a]	0.113	1.23	0.017(0.138)[a]	1.62(0.85)[a]
7	Caffeic acid	2.2	10.01	0.200	2.00	0.015	0.96	0.203	2.03	0.015	0.94
8	Syringic acid	2.2	11.74	0.221	1.88	0.017(0.085)[a]	1.07(0.81)[a]	0.218	1.86	0.015(0.067)[a]	0.98(0.63)[a]
9	(−)-Epicatechin	18.2	13.22	0.231	1.75	0.023(0.028)[a]	0.94(0.61)[a]	0.224	1.70	0.019(0.159)[a]	0.81(0.42)[a]
10	Syringaldehyde	3.4	14.89	0.210	1.41	0.020	1.16	0.236	1.59	0.018	1.06
11	*p*-Coumaric acid	1.8	16.08	0.236	1.47	0.020	1.09	0.227	1.41	0.019	1.01
12	Ferulic acid	2.8	19.35	0.242	1.25	0.025	1.19	0.231	1.19	0.021	1.00
13	*trans*-Resveratrol	2.0	25.07	0.114	0.46	0.024(0.029)[a]	1.33(0.68)[a]	0.089	0.35	0.019(0.026)[a]	1.08(0.62)[a]
14	Myricetin	3.0	26.23	0.101	0.39	0.041	1.31	0.078	0.30	0.039	1.24
15	Quercitrin	11.8	28.16	0.092	0.33	0.006	1.74	0.067	0.24	0.005	1.58
16	Quercetin	8.0	29.02	0.098	0.34	0.040	3.90	0.067	0.23	0.022	2.16
17	Kaempferol	7.6	31.52	0.108	0.34	0.076	4.38	0.065	0.21	0.031	1.76
			n[b]=3×5					n=5			

[a] Fluorimetric detection.
[b] Five replicates in three different days. SD, standard deviation; and RSD, relative standard deviation.

Table 3
Parameters of the calibration of polyphenolic compounds

No.	Compound	Absorbance						Fluorescence				
		Equation	Linear range (mg/l)	SD[a]	R^2	LOD[b]	LOQ[c]	Equation	SD[a]	R^2	LOD[b]	LOQ[c]
1	Gallic acid	$y=0.681\times10^5x-0.008\times10^5$	0.7–7.2	1485	0.999	0.06	0.19					
2	Protocatechuic acid	$y=0.381\times10^5x-0.010\times10^5$	1.0–10.4	662	0.999	0.12	0.39					
3	Protocatechuicaldehyde	$y=1.002\times10^5x+0.005\times10^5$	0.6–6.4	87	0.999	0.03	0.10					
4	(+)-Catechine	$y=0.140\times10^5x+0.021\times10^5$	3.4–33.6	441	0.999	0.11	0.36	$y=1.969\times10^5x-0.131\times10^5$	1773	0.999	0.093	0.31
6	Vanillic acid	$y=0.375\times10^5x+0.008\times10^5$	0.5–4.8	833	0.998	0.07	0.23	$y=6.557\times10^5x-0.395\times10^5$	13748	0.999	0.068	0.22
7	Caffeic acid	$y=0.701\times10^5x-0.007\times10^5$	0.4–4.4	818	0.999	0.06	0.20					
8	Syringic acid	$y=0.692\times10^5x+0.004\times10^5$	0.4–4.4	1929	0.995	0.05	0.16	$y=4.792\times10^5x+0.094\times10^5$	8362	0.999	0.003	0.01
9	(−)-Epicatechin	$y=0.127\times10^5x-0.007\times10^5$	3.6–36.4	110	0.999	0.34	1.13	$y=1.984\times10^5x-0.011\times10^5$	1695	0.999	0.031	0.10
10	Syringaldehyde	$y=0.448\times10^5x+0.015\times10^5$	0.7–6.8	520	0.999	0.04	0.13					
11	*p*-Coumaric acid	$y=0.964\times10^5x+0.175\times10^5$	0.4–4.0	2832	0.995	0.08	0.26					
12	Ferulic acid	$y=0.766\times10^5x-0.021\times10^5$	0.5–5.6	975	0.998	0.08	0.27					
13	*trans*-Resveratrol	$y=0.827\times10^5x+0.074\times10^5$	0.4–4	909	0.999	0.02	0.07	$y=1.877\times10^5x+0.045\times10^5$	829	1.000	0.003	0.01
14	Myricetin	$y=0.998\times10^5x-0.019\times10^5$	0.6–6.0	1565	0.998	0.06	0.20					
15	Quercitrin	$y=0.026\times10^5x-0.004\times10^5$	2.5–23.6	57	0.998	0.51	1.60					
16	Quercetin	$y=0.091\times10^5x+0.046\times10^5$	1.6–16	416	0.984	0.54	1.50					
17	Kaempferol	$y=0.142\times10^5x+0.066\times10^5$	1.5–15.2	488	0.992	0.21	0.70					

[a] SD, estimated standard deviation.
[b] LOD, detection limit (mg/l).
[c] LOQ, determination limit (mg/l).

tor was lower than 1.50 and 0.85% between and within run, respectively. Besides, the application of *t*-tests showed that there is not a statistically significant difference (P value>0.05) between the concentrations obtained between-day and within-day at the 95.0% confidence level. The limit of detection was calculated as the analyte concentration giving a signal 3 times as high as the blank value and is in the range 0.02 mg/l for *trans*-resveratrol to 1.51 mg/l for quercitrin with absorbance detection, while it is in the range from 0.003 mg/l for epicatechin and *trans*-resveratrol to 0.093 mg/l for catechin with fluorescence detection.

Fig. 2 shows chromatograms of a directly injected sample of red wine using both detectors. It can be observed that the sensitivity for the determination of the phenolic compounds when a wine sample was directly injected is lower and the chromatogram is more complex; however, although the fluorescence detector allowed the identification of three compounds in the mixture, most compounds cannot be determined, which makes necessary a prior extraction step to get the advantages of the use of both detectors in series. Chromatograms in Fig. 3 show that when the developed extraction procedure used the efficiency, selectivity and sensitivity are higher and the complexity of the chromatogram was reduced, allowing to identify compounds after spiking samples with standards. It can be seen that epicatechin and syringaldehyde peaks are overlapped in the absorbance mode epicatechin cannot be quantified,

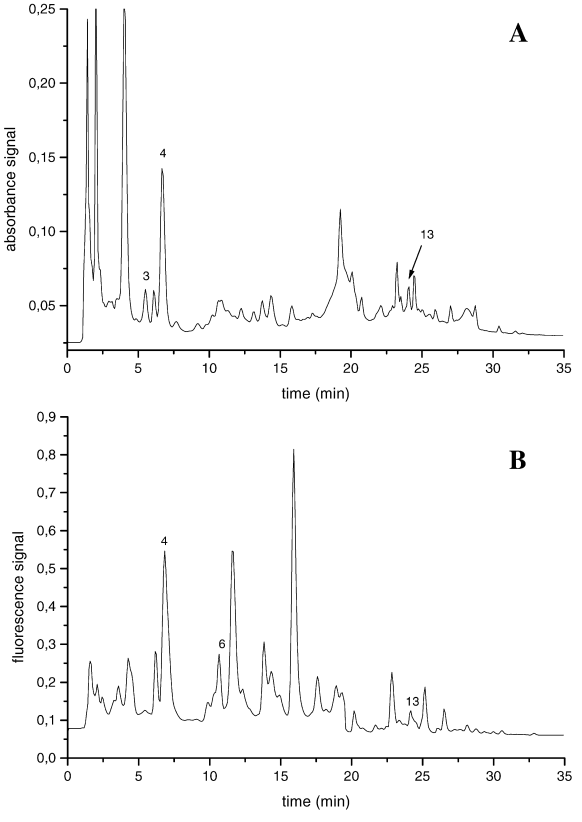

Fig. 2. Chromatograms of directly injected sample of red wine using: (A) absorbance detection (λ=280 nm), (B) fluorescence detection (λ_{ex}=278 nm and λ_{em}=360 nm over 17.5 min and λ_{ex}=330 nm and λ_{em}=374 nm for 16.5 min). Chromatographic conditions as for Fig. 1.

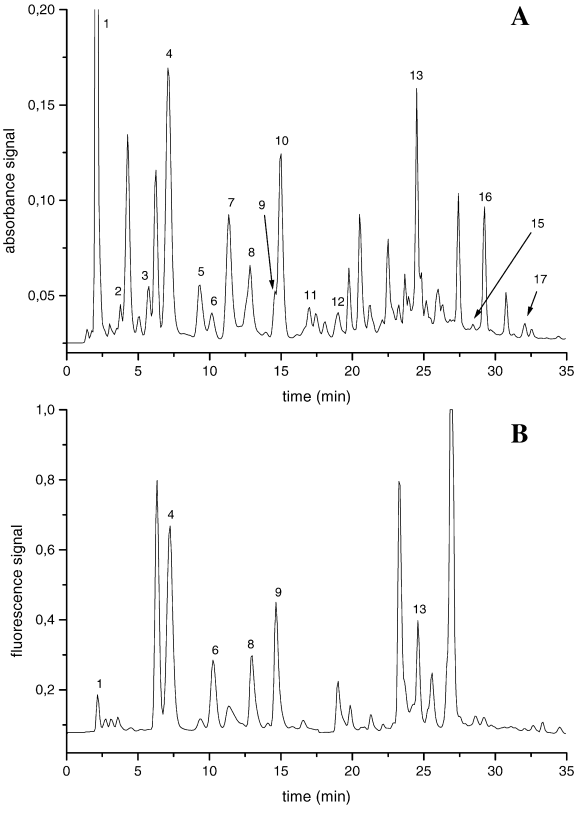

Fig. 3. Chromatograms of a red wine sample (5 ml) injected after a liquid–liquid extraction procedure extracting with 5 ml of diethyl ether for 20 min and solved methanol–water (1:1, v:v) using: (A) absorbance detection (λ=280 nm), (B) fluorescence detection (λ_{ex}=278 nm and λ_{em}=360 nm over 17.5 min and λ_{ex}=330 nm and λ_{em}=374 nm for 16.5 min). Chromatographic conditions as for Fig. 1.

but the fluorescence detector gives higher selectivity as this peak appears well resolved and with an increase sensitivity of at least 16 times higher. Another compound appear more resolved in the fluorescence detector although the increasing of sensitivity not is as high, reducing the complexity of the chromatogram. Fig. 3B also shows that other highly fluorescent polyphenolic compounds appear in the sample which are not present in the standard solution, so the fluorescence detector in series with the UV–Vis detector can be a potent tool to increase selectivity and sensitivity for the determination of phenolic compounds. Fig. 4 shows the chromatogram of a white wine, and although the amount of these polyphenolic compounds is lower, the use of an extraction procedure in combination with both detectors allows identifying and quantifying all compounds. Concentrations of some polyphenolic compounds found in the red and white wine samples are included in Table 4. Gallic acid, (+)-catechin, (−)-epicatechin and quercetin are present higher concentrations in the red wine samples. *trans*-Resveratrol content is similar all red wine samples with an average value of 3.00 ppm.

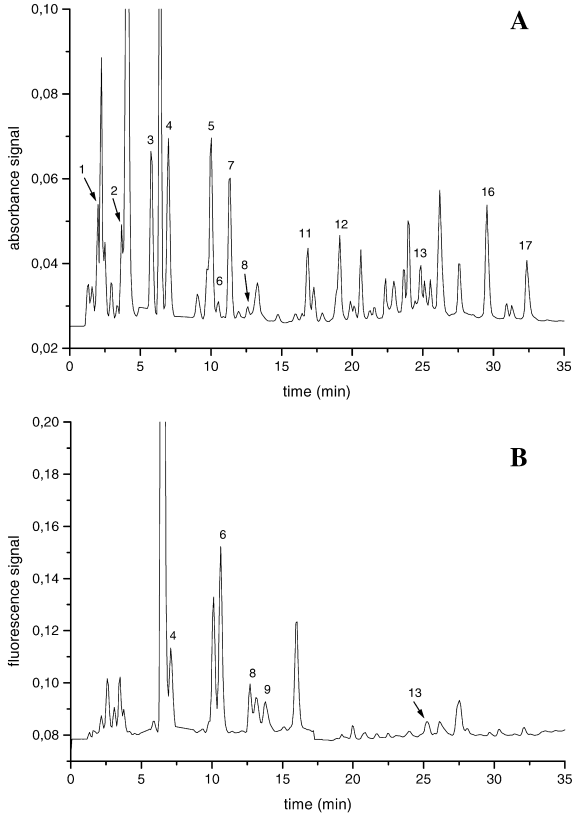

Fig. 4. Chromatograms of white wine sample injected after a liquid–liquid extraction procedure as for Fig. 3 using: (A) absorbance detection, (B) fluorescence detection. Detection and chromatographic conditions as for Fig. 3.

A discrepancy can be seen in Table 4 for (+)-catechin concentrations as quantified by both detectors. However comparing the absorbance and fluorescence spectra of the wine samples with the corresponding peak for (+)-catechin standard solution show that its peak in wine samples is overlapped with an unknown non-fluorescent compound. Thus, fluorescence peak quantitation is much better for (+)-catechin. It is well justified the selectivity of the fluorimetric detection is very high and it is possible to quantify peaks that appears as overlapped peaks in the chromatograms when a UV–Vis detector is used. For (−)-epicatechin appears too as an overlapped peak when UV–Vis detection is used, so its quantitation was realised with fluorescence detection. In the case of *trans*-resveratrol the results are similar using both detectors, but results are better when using the fluorescence detector as excitation and emission wavelength are the same as for the standard which allows to obtain a maximum sensitivity while with the absorbance detector a compromise wavelength has to be used

On comparing results for red and white wines it can be seen that concentrations are lower in white wines except for *p*-coumaric acid and kaempferol. Pazourek et al. [30] have found similar results for *p*-coumaric acid concentration in Canary Islands red and white wine samples. *trans*-Resveratrol content is about 30 times lower in white wine samples than in red wine samples, which shows that the higher sensitivity of the fluorimetric detection is very useful to determine this and another minor components in wine samples, specially in white wine samples.

4. Conclusions

A method for analysis of 16 phenolic compounds in wine samples by HPLC using fluorescence and absorbance detectors in series is presented. The increasing of sensitivity of several compounds in this

Table 4
Polyphenolic content in red and white wine samples

No.	Compound	Wine 1		Wine 2		Wine 3		Wine 4		Wine 5		White wine	
		c (mg/l)	*c* (mg/l)[a]	*c* (mg/l)	*c* (mg/l)[a]	*c* (mg/l)	*c* (mg/l)[a]	*c* (mg/l)	*c* (mg/l)[a]	*c* (mg/l)	*c* (mg/l)[a]	*c* (mg/l)	*c* (mg/l)[a]
1	Gallic acid	26.78		43.09		34.80		41.10		15.13		0.54	
2	Protocatechuic acid	0.55		–		1.10		0.37		–		0.58	
3	Protocatechuicaldehyde	0.60		0.68		1.17		0.87		0.62		1.04	
4	(+)-Catechine	104.51	31.69	112.25	23.73	118.85	25.57	132.04	26.79	102.22	10.66	17.86	0.70
6	Vanillic acid	1.12	1.63	1.00	1.51	1.58	2.11	1.11	1.58	0.80	1.22	0.17	0.41
7	Caffeic acid	4.42		2.80		3.41		2.95		2.12		1.58	
8	Syringic acid	2.36	2.19	2.69	2.61	2.49	1.93	2.73	2.64	1.04	1.64	0.08	0.06
9	(−)-Epicatechin	–	19.77	–	8.93	–	7.25	–	9.44	–	7.04	–	0.51
10	Syringaldehyde	7.11		21.36		12.62		24.05		44.51		–	
11	*p*-Coumaric acid	0.11		0.27		0.13		0.30		0.27		0.22	
12	Ferulic acid	0.61		0.68		0.78		0.77		0.55		0.39	
13	*trans*-Resveratrol	2.70	3.69	2.29	3.64	1.36	2.90	2.56	3.76	1.45	1.90	0.14	0.13
14	Myricetin	–		–		0.56		–		–		–	
15	Quercitrin	–		–		3.52		–		2.56		–	
16	Quercetin	17.00		15.70		2.88		17.35		21.06		8.35	
17	Kaempferol	0.68		1.05		0.57		1.41		2.37		2.69	

[a] Fluorimetric detection; *c*, concentration.

mixture reached using the fluorescence detector allow to quantify these compounds without any modification in the gradient profile. The coupling of absorptiometric and fluorimetric detectors in series allows to discriminate between fluorescent and non-fluorescent overlapping peaks, besides it may be use to identify and quantify other fluorescent compounds present in wine samples.

Acknowledgements

We acknowledge financial support of this work by the University of La Laguna Precompetitive Research Project No. 243-84/97. S.M. thanks the Socrates–Erasmus Program, University of La Laguna–Masaryk University.

References

[1] V.L. Singleton, P. Esau, in: Phenolic Substance in Grapes and Wine and Their Significance, Academic Press, New York, 1969.
[2] A.G. Lea, P. Bridle, C. Timberlake, V.L. Singleton, Am. J. Enol. Vitic. 30 (1979) 289.
[3] F. Shahidi, M. Naczk, in: Food Phenolics — Sources, Chemistry, Effects, Applications, Technomic, Philadeplhia, PA, 1995.
[4] A.W. Jaworski, C.Y. Lee, J. Agric. Food Chem. 35 (1987) 257.
[5] E.H. Siemann, L.L. Creasy, Am. J. Enol. Vitic. 43 (1992) 49.
[6] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, Ch.W.W. Beecher, H.H.S. Fong, N.R. Farnsworth, A.D. Kimghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Science 275 (1997) 218.
[7] A.A. Bertelli, Int. J. Tiss. React. 17 (1995) 1.
[8] D.M. Goldberg, J. Yan, E. Ng, E.P. Diamandis, A. Karumanchiri, G. Soleas, A.L. Waterhouse, Am. J. Enol. Vitic. 46 (1995) 159.
[9] B.Y. Ong, C.W. Nagel, J. Chromatogr. 157 (1978) 345.
[10] L.W. Wulf, C. Nagel, J. Chromatogr. 116 (1976) 271.
[11] V. Cheynier, J. Rigaud, M. Moutounet, J. Chromatogr. 472 (1989) 428.
[12] G.P. Cartoni, F. Coccioli, L. Pontelli, J. Chromatogr. 537 (1991) 93.
[13] D.A. Guillén, C.G. Barroso, J.A. Pérez-Bustamante, J. Chromatogr. A 655 (1993) 227.
[14] D.A. Guillén, C.G. Barroso, J.A. Pérez-Bustamante, J. Chromatogr. A 724 (1996) 117.
[15] C. García-Viguera, P. Bridle, Food Chem. 54 (1995) 349.
[16] O. Maman, F. Marseille, B. Guillet, J. Disnar, P. Morin, J. Chromatogr. A 755 (1996) 89.
[17] L. Arce, M.T. Tena, A. Rios, M. Valcárcel, Anal. Chim. Acta 359 (1998) 27.
[18] A.J. Zemann, J. Cap. Electrophoresis 2 (1995) 131.
[19] M.A. Rodríguez-Delgado, M.L. Pérez, R. Corbella, G. González, F.J. García-Montelongo, J. Chromatogr. A 871 (2000) 427.

[20] D.M. Goldberg, A. Karumanchiri, E. Ng, J. Yan, E.P. Diamandis, G. Soleas, Am. J. Enol. Vitic. 43 (1995) 281.
[21] D.M. Goldberg, J. Yan, E. Ng, E.P. Diamandis, A. Karumanchiri, G. Soleas, A.L. Waterhouse, Anal. Chem. 66 (1994) 3959.
[22] G.P. Cartoni, F. Coccioli, R. Jasionowska, J. Chromatogr. A 709 (1995) 209.
[23] D.A. Guillén, E. Bru, C.G. Barroso, J.A. Pérez-Bustamante, Quím. Anal. 15 (1996) 58.
[24] R.D. Salagoity-Aguste, A. Bertrand, J. Sci. Food. Agric. 35 (1984) 1241.
[25] R. Di Stefano, E. García Moruno, Vignevini 11 (1986) 37.
[26] N. Simpson, Int. Chromatogr. Lab. 11 (1992) 13.
[27] M.A. Rodríguez-Delgado, S. Malovaná, J.P. Pérez, F.J. García Montelongo, Anal. Chim. Acta 428 (2001) 245.
[28] R. Pezet, V. Pont, P. Cuenta, J. Chromatogr. A 663 (1994) 191.
[29] P. Viñas, C. López-Erroz, J.J. Marín-Hernández, M. Hernández-Cordoba, J. Chromatogr. A 871 (2000) 85.
[30] J. Pazourek, G. González, A.L. Revilla, J. Havel, J. Chromatogr. A 874 (2000) 111.