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Determination of *trans*-resveratrol and other polyphenols in wines by a continuous flow sample clean-up system followed by capillary electrophoresis separation

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Abstract

A new method coupling flow injection (FI) with capillary electrophoresis (CE) has been developed using diode array detection to measure the concentration of *trans*-resveratrol in wines, in particular because of the interest in its biological properties and cancer prevention. A FI system furnished with a C-18 minicolumn was used to clean up the wines by solid phase extraction prior to CE. The analytes were eluted from C-18 by using methanol and then driven from the FI system to the autosampler of the CE equipment by a programmable arm. The 3σ detection limit ranged from 0.05 mg l^{-1} (*trans*-resveratrol) to 0.36 mg l^{-1} [(-)epicatechin]. The recoveries of added *trans*-resveratrol and other polyphenols from synthetic wines were between 92% to 110%, (mean of 99%). The method is faster and simpler than those previously reported which used liquid–liquid extraction and liquid chromatography. © 1998 Elsevier Science B.V.

Keywords: Capillary electrophoresis; Flow system; *Trans*-resveratrol; Solid phase extraction

1. Introduction

Research on the *trans*-resveratrol content in wines has been due to an interest in the prevention of cancer and heart disease by ingestion of chemical agents that reduce the risk of carcinogenesis [1–3]. Resveratrol inhibits cellular events associated with tumour initiation, promotion and progression. The compound also functions as an antimutagen and has anti-fungal properties. Resveratrol has been found in at least 72 plant species, a number of which are components of the human diet. In *vinifera* varieties, the resveratrol con-

tent of whole berries steadily declines between the green stage and complete maturity, nearing zero in ripe fruit. On the other hand, resveratrol synthesis was found to be located in the skin, thus showing that the principal resistance of grapes to fungal attacks takes places at this level [4]. *Trans*-resveratrol concentrations of wines show marked fluctuations which seem to be temperature dependent. Wines from Italy and Spain which are subject to warmer and drier conditions tend to have low *trans*-resveratrol concentrations [5,6].

Many recent papers [3–15] describing methods to assay the content of *trans*-resveratrol in wines have been based on liquid or gas chromatographic techniques (LC or GC). Capillary electrophoresis (CE) can

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be used as an alternative to LC in the determination of resveratrol, exploiting the high-resolution separation achieved by the different migration modes, viz. capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC). Because of their acidity, phenols can be determined as anions under CZE conditions or as either anions or uncharged species under MEKC conditions. Methods to determine phenolic compounds have been developed by several authors [16–21] using the CE technique, but only three of them have determined polyphenols in wines [16–18]. Some combined flow injection (FI)–CE systems have been described in previous papers [22–24] but in this work, wines were first analyzed for their content of one specific phenolic compound (*trans*-resveratrol) by FI–CE, which has not been done previously. Secondly, studies were also carried out to determine polyphenols in wines.

The determination of polyphenolic compounds in wine generally requires the use of extraction and preconcentration techniques prior to CE, in order to simplify the electropherograms. This is because many polyphenolic compounds in wine are present only in very low concentrations and wine matrices are highly complex. Firstly, liquid–liquid extraction procedures have been proposed for sample preparation [16,17]. More recently, solid phase extraction (SPE) has been applied as an alternative to liquid–liquid extraction [10,13,25]. In this paper, on-line SPE using C-18 prior to the CE separation is proposed for the extraction/preconcentration of these compounds in wine, providing lower detection limits and avoiding the interferences from other compounds. The proposed method is simple and reduces sample manipulation.

2. Experimental

2.1. Reagents

Standard and buffer solutions were prepared in purified water (18 m Ω) by using a Millipore Milli-Q water purification system. A 0.1 M sodium borate buffer solution of pH 9.5 was also used.

The phenolic compounds were *trans*-resveratrol, (–)epicatechin, (+)catechin, gentic acid, salicylic acid, myricetin, quercetin, *p*-coumaric acid, caffeic acid and gallic acid, all supplied by Sigma. A stock

standard solution of 1000 mg l^{–1} of each compound was prepared in methanol and stored at –4°C in dark conditions. Working standard solutions were prepared by diluting the stock standard solutions with purified water or in a synthetic matrix of wine. C-18 and SAX were used as the solid phase, and methanol was used as the eluent. Solutions of 0.1 and 0.5 M sodium hydroxide were used for conditioning the capillary. A synthetic wine matrix was prepared with ethanol, tartaric acid and citric acid. All these chemicals were supplied by Merck.

2.2. Apparatus

Beckman P/ACE 5500 capillary electrophoresis equipment provided with a diode array detector and fused-silica capillary (75 mm internal diameter and 67 cm long) was used for the separation and quantification of the analytes. A Gilson Minipuls-3 peristaltic pump, four Rheodyne 5041 injection and selection valves, and PTFE tubing of 0.5 mm i.d. were used to set up the manifold. The continuous filtration system was developed in our laboratory and tested with different types of microfilters from Millipore (pore size 0.8, 0.45 and 0.22 mm). A home-made programmable arm [26] controlled by a microcomputer through an electronic interface was used in order to automate the introduction of the sample after the prior treatment in the FI system.

An Uvatrom-70 UV lamp was used to irradiate the sampler. The minicolumn for the preconcentration and extraction of polyphenols was a 50 mm length \times 2 mm i.d. tube containing octadecylsilane (C-18) packed between two glass-fibre pads (2 mm thick). The ends of the column were fitted with flanged pieces of 0.5 mm i.d. manifold tubing.

2.3. Operating conditions

Similar CE conditions to those reported previously by Gil et al. [18] were used, and afterwards optimised to this particular method. The running buffer was 0.1 M sodium borate (pH 9.5) with a voltage of 20 kV, average current of 97 μ A and temperature of 20°C. Samples were injected by hydrodynamic injection for 5 s. Electropherograms were recorded at 280 nm. The separation was carried out from the positive to the negative electrode. The capillary was

conditioned daily by washing with methanol (5 min) followed by freshly prepared 0.5 M sodium hydroxide (5 min), 0.1 M sodium hydroxide (5 min), ultrapure water (5 min) and fresh buffer (5 min). In order to optimise the migration time and the peak shape reproducibility, the capillary was flushed between analyses with 0.1 M sodium hydroxide (2 min), ultrapure water (2 min) and fresh buffer (2 min).

2.4. Wine samples

A group of commercially available wines from different Spanish regions and from other countries (Argentina, France) was analyzed. However, *trans*-resveratrol was not found in any of the wines analyzed in our laboratory, and consequently spiked samples with levels of concentrations in the same range as those found in the literature [3,5,18] were used.

Synthetic samples were prepared by mixing different phenolic compounds dissolved in a matrix containing tartaric acid, citric acid, acetic acid and ethanol in similar concentrations to those detailed in real wine samples.

2.5. Sample preparation

The off-line trace enrichment process was carried out using 500 mg C-18 cartridges (Varian). Extraction was carried out using a Bond Elut/Vac Elut system. Different volumes of sample (1–10 ml) were passed through the cartridge and the phenolic compounds retained were eluted with different volumes of methanol (1–10 ml). Wine samples were filtered through a 0.45 mm filter before preconcentration.

The on-line trace enrichment process was carried out using a FI system coupled to CE equipment. In Fig. 1(a) and (b), the selection valve SV₁ allowed the sequential introduction of calibration solutions and wines samples, which were continuously filtered before their introduction into the manifold. Selection valve SV₂ allowed the sample to be driven to the autosampler of the CE equipment through the programmable arm (channel 2) or to waste (channel 1). When preconcentration was needed (Fig. 1(a)), valve IV₁ was switched to the filling position, allowing the sample to pass through the C-18 minicolumn (previously conditioned with methanol and water), where polyphenols were preconcentrated. In this position an

appropriate volume of sample was passed through the column in order to retain sufficient amounts of analytes to obtain sensitive signals by CE afterwards. When the preconcentration was complete (Fig. 1(b)), 2 ml of methanol was introduced through valve SV₃ to elute the analytes from the column, the stream now flowing in the opposite direction with respect to the preconcentration step. This volume of eluent was found to be enough to elute quantitatively the analytes retained at the concentration levels present in the wine samples. Thus, a substantial reduction of the dispersion of the analytes in the flowing stream was achieved. The entire fraction containing the eluted analytes was driven to a microvial of the CE sampler via the activation of the programmable arm which is controlled by the CE software. The time taken by the sample to reach the CE equipment was measured using a dye (methylene blue). While the analysis is performed in the CE instrument, a new sample is processed in the flow system.

3. Results and discussion

Peaks in the electropherograms of wine extracts were identified as *trans*-resveratrol and other polyphenols by comparison of their spectra and retention times with ethanolic standard solutions of these compounds. Fig. 2 demonstrates the satisfactory resolution accomplished for the major peaks of interest when the eluent was monitored at 280 nm. The different phenolic compounds were separated according to their charge to mass ratio at pH 9.5, and their chemical structures are shown in Table 1. *Trans*-resveratrol, γ (-)-epicatechin, (+)-catechin, gentisic acid, salicylic acid, myricetin, quercetin, *p*-coumaric acid, caffeic acid and gallic acid were tested; good separations were achieved, except that the peak of myricetin overlapped with that of quercetin. The electro-osmotic flow from anode to cathode is the main driving force under these conditions and causes the solutes to be driven to the cathode, even though they are negatively charged.

As it can be seen in Fig. 2, the determination of resveratrol in wines is faster by CE than by LC (typical analysis times of 10 min in CE, and at least 25 min in LC [3,4,21]). In addition, a more efficient clean up of wine samples is required in LC in order to avoid

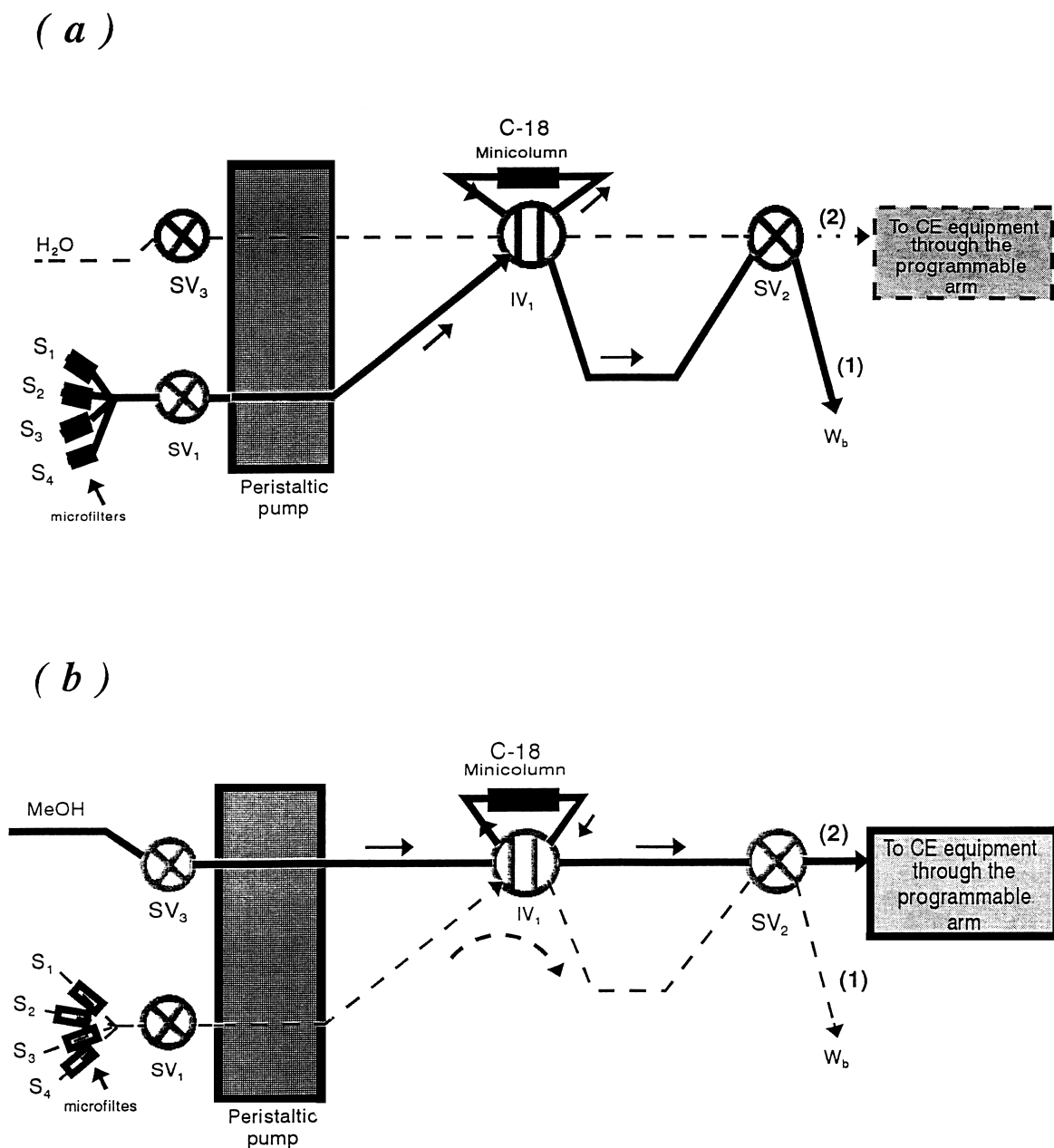


Fig. 1. FI manifold used for sample treatment and its introduction into the CE equipment. (IV=injection valve; SV=selection valve; w=waste; S=wine samples): pre-concentration (a) and elution (b) steps, (for detail, see text).

column damage and thus maintaining the column performance. Selectivity is higher in CE than LC, and the electropherogram of a wine sample showed

less peaks than the chromatogram of the same sample. Furthermore, better peak resolution was achieved in CE than in LC for charged analytes.

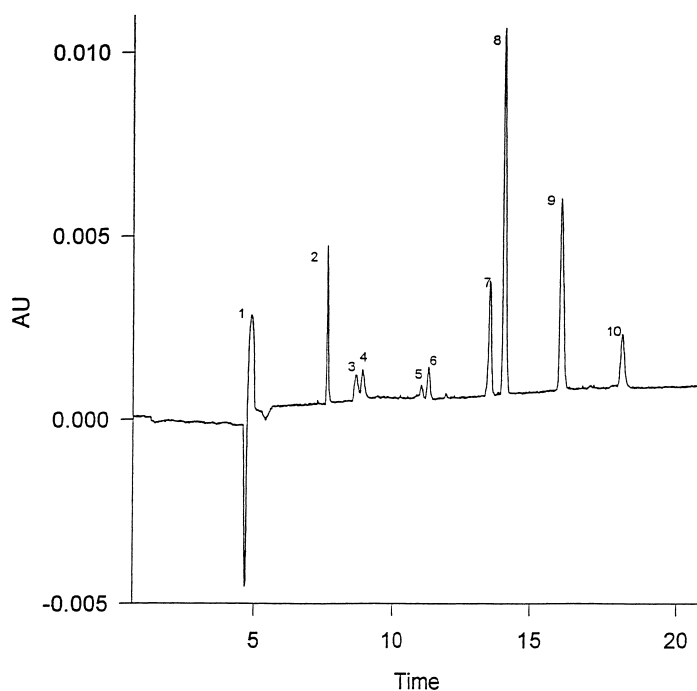


Fig. 2. Electropherogram of a standard mixture of polyphenols: 1=EOF; 2=*trans*-resveratrol; 3=(-)epicatechin; 4=(+)catechin; 5=genticic acid; 6=salicylic acid; 7=quercetin and myricetin; 8=*p*-coumaric acid; 9=caffeic acid; 10=gallic acid. AU=absorbance units.

3.1. Solid-phase extraction

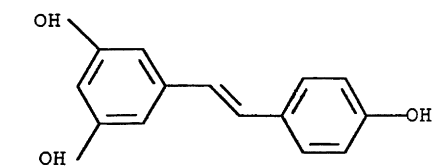
The method was first applied to the analysis of wines which had not been subjected to any special treatment. In this case, they were only filtered through a 0.45 mm nylon membrane. In order to decrease the matrix effect and to determine the resveratrol and other polyphenols at low concentrations, different solid-phase extraction minicolumns were tested. C-18 and strong anionic exchangers provided the best results, but problems relating to incomplete elution of analytes were observed when exchanger materials were used. Fig. 3(a) and (b) shows the electropherograms resulting from the analysis of a red wine spiked with 1 mg l^{-1} polyphenols. Fig. 3(a) shows that gallic acid, caffeic acid, *p*-coumaric acid and quercetin can be detected by direct injection of the spiked wine. In the range 5–10 min, no polyphenols could be identified because spectral peak purities were below 90%. Fig. 3(b) shows the result of the optimized SPE treatment applied to the same wine. As can be seen, by using SPE the first part of the electropherogram shows

less peaks and resveratrol was eluted in a cleaner zone. In comparison to the liquid–liquid extraction procedure commonly used as sample preparation for the determination of polyphenols in wines, the proposed SPE process is simpler, faster, more efficient, easier to automate, and cleaner from an environmental point of view.

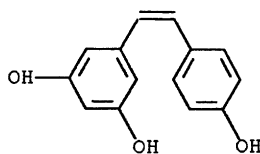
Variables such as the volume of organic solvent to desorb the analytes, the volume of sample and FI variables were studied in order to simultaneously clean-up the wine samples and concentrate *trans*-resveratrol and other phenolic compounds by SPE using a C-18 minicolumn.

The volume of methanol used to elute polyphenols from C-18 cartridges was the first parameter investigated in the optimization of these variables. Different volumes (1–5 ml) of synthetic samples of *trans*-resveratrol, at different concentrations, were passed through the C-18 cartridge to retain the analytes. Then different volumes of methanol were passed through the minicolumn to elute these compounds. The best results were obtained when 2 ml of both wine and

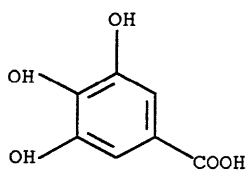
Table 1
Structures of the compounds studied in this work



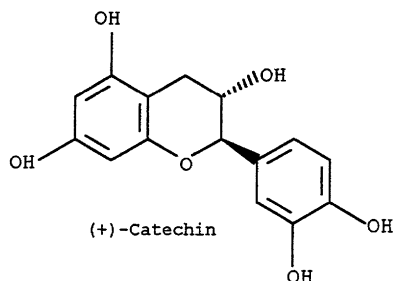
trans-Resveratrol



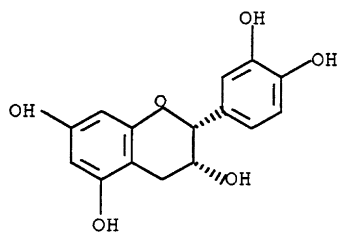
cis-Resveratrol



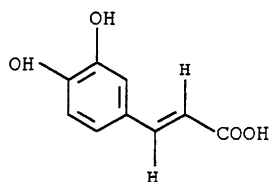
Gallic Acid



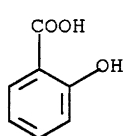
(+)-Catechin



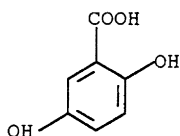
(-)-Epicatechin



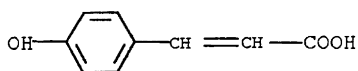
Caffeic Acid



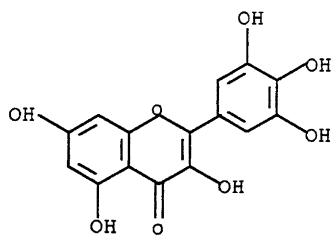
Salicylic acid



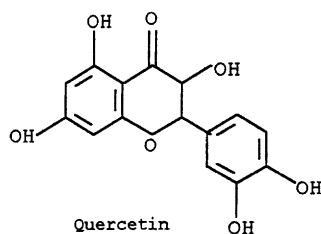
Gentisic Acid



p-Coumaric acid



Myricetin



Quercetin

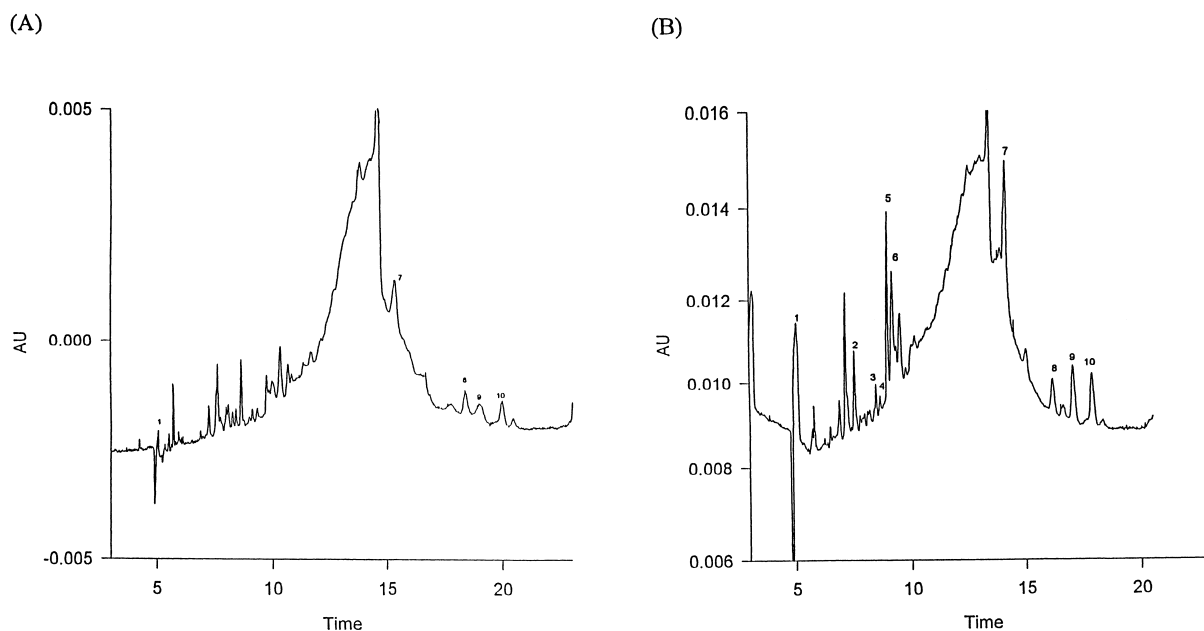


Fig. 3. Electropherograms showing the clean-up and preconcentration effect of the SPE procedure proposed: direct injection of a spiked wine sample (a) and the same spiked wine sample after SPE (b) 1=EOF; 2=*trans*-resveratrol; 3=(-)epicatechin; 4=(+)catechin; 5=genticic acid; 6=salicylic acid; 7=quercetin; 8=*p*-coumaric acid; 9=caffeic acid; 10=gallic acid. AU=arbitrary units.

Table 2
Recoveries obtained for *trans*-resveratrol by using octadecylsilane C-18 for SPE

Concentration (mg l ⁻¹)	Sample volume/MeOH volume(ml)	Recovery (%)
5.0	1/1	81
	2/2	100
	4/4	98
	5/5	90
1.0	1/1	61
	2/2	99
	4/4	98
0.5	1/1	67
	2/2	99.5
	4/4	97
	5/5	95

methanol were used. Poor recoveries were obtained when smaller volumes were used, whereas higher volumes only diluted the sample (see Table 2).

The optimum values for the hydrodynamic variables of the FI system were as follows. A sample

volume of 5 ml (flowing at 4 ml min⁻¹) was enough to achieve the sensitivity required for the polyphenols that needed preconcentration. The elution was performed with 2 ml of methanol. The preconcentration time was 6 min, whereas a time of 30 s was needed for the elution step. During this time some of the polyphenols can be electrophoretically determined in the CE equipment when no preconcentration was required. To achieve the maximum precision of the results, the C-18 minicolumn was changed every day. Furthermore, to make sure of the clean-up of the minicolumn after the treatment of 20 wine samples, a blank was analyzed using the same column.

3.2. Performance of the method

The calibration graphs were produced from results obtained by injecting standard solutions in the range 0.05–100 mg l⁻¹. Each point of the calibration graph corresponded to the mean value obtained from three independent area measurements. The limit of detection (LOD) was calculated from the blank value plus 3 times its standard deviation, whereas limit of quanti-

Table 3

Figures of merit of the proposed method for the determination of phenolic compounds (hydrodynamic injection)

Analyte	$y=a+bx$	$S_{y/x}$	r	R	r.s.d. (%)	LOD	LOQ
<i>trans</i> -Resveratrol	$a=-0.014\pm 5.2\times 10^{-3}$ $b=0.302\pm 3.7\times 10^{-3}$	0.013	0.999	99.88	4.8	0.05	0.17
(-)-Epicatechin	$a=0.159\pm 0.29$ $b=2.410\pm 0.11$	0.644	0.992	98.33	7.1	0.36	1.20
(+)-Catechin	$a=0.068\pm 1.2\times 10^{-3}$ $b=0.151\pm 7.7\times 10^{-3}$	0.024	0.992	98.46	5.9	0.24	0.80
Gentisic acid	$a=-0.047\pm 2.8\times 10^{-2}$ $b=0.314\pm 9.5\times 10^{-3}$	0.048	0.997	99.45	6.3	0.27	0.90
Salicylic Acid	$a=-1.904\pm 5.0\times 10^{-3}$ $b=0.052\pm 1.7\times 10^{-3}$	0.008	0.997	99.35	3.7	0.30	0.90
Quercetin	$a=-0.175\pm 0.15$ $b=1.378\pm 5.5\times 10^{-2}$	0.320	0.994	98.73	3.2	0.32	1.06
<i>p</i> -Coumaric acid	$a=0.021\pm 9.9\times 10^{-3}$ $b=0.200\pm 6.2\times 10^{-3}$	0.019	0.997	99.43	6.5	0.14	0.50
Caffeic acid	$a=0.053\pm 0.18$ $b=1.580\pm 6.8\times 10^{-2}$	0.394	0.993	98.54	6.3	0.34	1.14
Gallic acid	$a=-0.021\pm 7.9\times 10^{-3}$ $b=0.081\pm 2.6\times 10^{-3}$	0.013	0.996	99.37	3.8	0.30	0.97

a =intercept; b =slope; $S_{y/x}$ = standard deviation of residuals; R =the curve-fitting level (in percent) obtained by analysis of variance for the validation of the model; r.s.d.=relative standard deviation; LOD=Limit of detection and LOQ=Limit of quantification; LOD and LOQ in mg l^{-1} . If replicate analyses were carried out at 0.5 mg l^{-1} for each phenol.

fication (LOQ) was calculated from the blank value plus 10 times its standard deviation. The corresponding regression equation and other characteristic parameters for the determination of the phenolic compounds are shown in Table 3. Eleven replicate analyses were performed on synthetic samples, with a concentration of 0.5 mg l^{-1} for each of the eight phenols which were passed through the C-18 mini-column prior to elution with methanol, in order to evaluate the precision of the method for each of the compounds determined (see Table 3). About 50 samples can be run every 24 h, including the time needed for analytical calibration and for column washing.

3.3. Analysis of wine samples

It has been reported that when wine extracts were protected from light, the conversion of *trans*-resveratrol to the *cis*-form did not occur [3]. In order to establish the influence of UV irradiation on *trans*-resveratrol stability, aqueous solutions at three different concentrations were prepared. Standard solutions of *trans*-resveratrol were protected from light because

a decrease of the concentration was observed after some hours. Less than 5 min irradiation was insufficient to obtain the *cis*-isomer, whereas the results were constant above 10 min; 30% of *trans*-resveratrol was transformed to *cis*-resveratrol.

Synthetic samples and spiked red and white wines were prepared in order to evaluate the accuracy of the method. Excellent recoveries were obtained (see Table 4). The proposed method was applied to the direct determination of resveratrol and other polyphenolic compounds in four different wines (white and red). The different parts of the Table 5 shows the concentrations of some of the polyphenolic compounds found in the wine samples. In order to validate the method the addition standard method was used for the determination of these phenolic compounds. In all cases the application of the *t*-test for the slopes of the calibrations graphs showed no significant statistical differences. Consequently there is no evidence of systematic error affecting the determination of (-)epicatechin, (+)catechin, *p*-coumaric acid and caffeic acid in wine by the proposed method. Recoveries and final concentrations found by using the standard addition method are shown in Table 5(a)–(d). Other polyphenolic compounds, such as *trans*-

Table 4
Analysis of synthetic samples by the proposed method (mg l⁻¹)

	<i>trans</i> -Resveratrol	(-)-Epicatechin	(+)-Catechin	Gentisic acid	Salicylic acid	Quercetin	<i>p</i> -Coumaric acid	Caffeic acid
SAMPLE 1								
ADDED	0.2	2.0	5.0	1.0	1.0	3.0	2.0	5.0
FOUND	0.19±0.02	1.93±0.05	5.00±0.08	0.97±0.03	0.99±0.05	2.80±0.1	2.10±0.05	4.93±0.02
RECOVERY (%)	95±10	96.5±2.5	100±1.6	97±3	99±5	93.3±3.3	105±2.5	98.6±0.4
SAMPLE 2								
ADDED	0.5	3.0	5.0	4.0	3.0	2.0	1.0	3.0
FOUND	0.47±0.07	3.10±0.06	5.20±0.07	3.80±0.1	2.90±0.07	2.20±0.1	0.98±0.05	3.00±0.1
RECOVERY (%)	94±14	103±2	104±1.4	95±2.5	96.6±2.3	110±5	98±5	100±3.3
SAMPLE 3								
ADDED	1.0	2.0	3.0	4.0	5.0	1.0	1.0	1.0
FOUND	1.05±0.03	2.10±0.1	2.97±0.03	3.95±0.01	4.80±0.1	0.92±0.04	0.98±0.03	1.10±0.05
RECOVERY (%)	105±3	105±5	105±1	98.7±0.25	96±2	92±4	98±3	110±5
SAMPLE 4								
ADDED	0.4	4.0	4.0	3.0	0.5	3.0	3.0	3.0
FOUND	0.37±0.02	3.90±0.2	3.97±0.04	3.00±0.1	0.50±0.03	3.04±0.05	2.97±0.05	2.95±0.05
RECOVERY (%)	92±5	97.5±5	99.2±1	100±3.3	100±6	101.3±1.6	99±1.6	98.3±1.6
SAMPLE 5								
ADDED	0.8	1.0	1.0	1.0	0.5	1.0	0.5	2.0
FOUND	0.83±0.04	1.00±0.07	0.93±0.09	0.98±0.02	0.47±0.1	1.00±0.1	0.51±0.03	1.89±0.2
RECOVERY (%)	103.7±5	100±7	93±9	98±2	94±20	100±10	102±6	94.5±10

Results are Mean ±S.D. (n=3).

Table 5
Determination of (–)-epicatechin, (+)-catechin, *p*-coumaric acid, caffeic acid in real samples

Wine	Original concentration* (mg l ⁻¹)	Concentration added (mg l ⁻¹)	Concentration found (mg l ⁻¹)	Recovery (%)
(a) Determination of (–)-epicatechin in real samples				
Rioja	42.03	5	5.2	104±7.3
		10	10.3	103±7.3
		15	14.8	98.6±7
Montilla	5.96	5	4.9	98±6.9
		10	10	100±7.1
		15	15.3	102±7.2
Valdepeñas	47.2	5	5	100±7.1
		10	10.1	101±7.1
		15	14.9	99.3±70.5
Bordeaux	48.6	5	4.8	96±6.8
		10	10.3	103±7.3
		15	15.1	100.6±7.14
(b) Determination of (+)-catechin in real samples				
Rioja	52.5	5.0	4.8	96±6
		10.0	10.3	103±6
		15.0	15.1	101±6
Montilla	16	5.0	5.0	100±6
		10.0	10.2	102±6
		15.0	15.1	101±6
Valdepeñas	23.3	5.0	4.9	98±6
		10.0	10.2	102±6
		15.0	14.7	98±6
Bordeaux	30.3	5.0	5.1	102±6
		10.0	10.3	103±6
		15.0	15.2	101±6
(c) Determination of <i>p</i> -coumaric acid in real samples				
Rioja	26.1	5.0	5.2	104±7
		10.0	10.3	103±7
		15.0	15.0	100±6
Montilla	3.9	5.0	4.8	96±6
		10.0	9.9	99±6
		15.0	15.2	101±6
Valdepeñas	8.24	5.0	4.8	96±6
		10.0	10.2	102±7
		15.0	14.8	99±6
Bordeaux	2.94	5.0	5.0	100±6
		10.0	10.1	101±6
		15.0	14.7	98±6
(d) Determination of caffeic acid in real samples				
Rioja	26.05	5.0	4.7	94±6
		10.0	10.0	100±6
		15.0	14.8	99±6
Montilla	1.3	5.0	4.9	98±6
		10.0	9.8	98±6
		15.0	14.8	99±6
Valdepeñas	6.2	5.0	4.9	98±6
		10.0	9.9	99±7
		15.0	15.1	101±6
Bordeaux	1.26	5.0	5.0	100±6
		10.0	10.1	101±6
		15.0	14.7	98±6

* Concentration obtained by using the standard addition method.

Table 6
Determination of phenolic compounds in spiked white and red wines (mg l^{-1})

		Rioja	Valdepeñas	Montilla	Bordeaux
<i>trans</i> -Resveratrol	Added	0.05	0.1	0.08	0.20
	Found	0.06±0.03	0.12±0.06	0.07±0.05	0.2±0.3
	Recovery(%)	120	120	88	100
Gentisic acid	Added	12.0	22.0	5.0	30
	Found	11.9±1.6	23.8±2.3	4.8±0.5	31.5±2.3
	Recovery(%)	99	108	96	105
Salicylic acid	Added	2.0	4.0	6.0	8.0
	Found	2.3±0.6	4.6±0.6	5.8±1.2	8.2±1.6
	Recovery(%)	115	115	97	102
Quercetin	Added	1.5	2.5	3.5	4.5
	Found	1.6±0.4	2.8±0.2	3.3±0.7	4.7±0.5
	Recovery(%)	107	112	94	104

Values are mean ±S.D. ($n=3$).

resveratrol, gentisic acid, salicylic acid and quercetin, were not found in the available samples, so spiked samples were used in these cases. As can be seen in Table 6 the amount found for these compounds in different types of wines were acceptable. The worst recoveries was found for *trans*-resveratrol, due to the very small amount determined. Spectral peak purity for all the polyphenols in Tables 5 and 6 were checked in wine samples.

4. Conclusion

In this paper, a method is described for the extraction and determination of eight phenolic constituent of wine by FI–CE. The FI system works as a preparation sample unit with SPE. The extract was directly introduced from the FI system to the autosampler of the CE through a programmable arm. Although phenolic compounds have previously been determined in wines by CE, this is the first paper that proposes a method for measuring *trans*-resveratrol by CE with prior automation of the sample treatment. It could be concluded that CE is able to implement existing methods for wine analyses. The method developed allows resveratrol and other polyphenolics to be determined at low levels with detection limits between 0.05–0.36 mg l^{-1} , thus improving the detection limits for these polyphenols in wines. Linearity, recovery, precision and sensitivity were highly satisfactory. The analysis time was 30 min for all the phenolic compounds and only 10 min for resveratrol.

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