ELSEVIER

Journal of Chromatography A, 855 (1999) 515–527

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Liquid chromatographic–mass spectrometric determination of phenolic compounds using a capillary-scale particle beam interface

Achille Cappiello[a,*], Giorgio Famiglini[a], Filippo Mangani[a], Maria Careri[b], Paola Lombardi[b], Claudio Mucchino[b]

[a]*Istituto di Scienze Chimiche, Università di Urbino, Urbino, Italy*

[b]*Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università di Parma, Parma, Italy*

Received 9 March 1999; received in revised form 27 May 1999; accepted 3 June 1999

Abstract

A capillary-scale particle beam interface was used to detect 18 phenolic compounds in red wine samples. This technique allows reproducible, library searchable electron ionization spectra at only 1 μl/min mobile phase flow-rate for a sensitive detection of the analytes in complex matrices. The method makes use of a narrow bore, reversed-phase packed capillary column for sample separation. Detection limits were in the low picogram range for most compounds. Sensitivity and response linearity were evaluated for eight phenolic acids, which are often encountered in red wines. The phenolic compound composition was outlined in two red wines obtained using different aging processes.

Keywords: Interfaces; LC–MS; Wine; Food analysis; Phenolic compounds; Organic acids

1. Introduction

The use of electron ionization (EI) in liquid chromatography–mass spectrometry interfacing is, when possible, a valid approach for the undoubted determination of several analytes in different matrices. If the molecular mass and nature of the compounds of interest do not interfere with the requirements of EI (vapor pressure and thermal stability), reproducible and characteristics mass spectra are generated. This feature is not of secondary importance in several 'small' molecule applications, where the analytes can be easily identified and their spectra matched with those collected in the literature. A particle beam (PB) LC–MS interface is based on the initial formation of an aerosol from the mobile phase effluent which, after droplet desolvation, is converted into a beam of solute particles. The particles are vaporized by contact onto a heated surface inside a conventional EI ion source and the gas phase molecules are eventually ionized.

The authors have demonstrated [1–4] that reducing the mobile phase flow-rate is the key for a higher sensitivity and an improved overall performance of the PB interface. As recently reported [4], the solvents used in the mobile phase can heavily affect several steps of the interfacing process with, sometimes, disappointing or unexpected results. Even the final vaporization of dry particles keeps trace of the initial aerosol conditions and the extent of adverse phenomena in the ion source, such as thermal decomposition and adsorption, may vary according-

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00708-6

ly. This is particularly true when the separation is carried out in reversed-phase conditions where water is almost obligatory in the mobile phase. Water has the highest boiling point and highest polarity of the solvents normally employed in HPLC. When the aerosol droplets generated in the nebulizer travel along the desolvation chamber, water is the last solvent to be removed from the solute particles. When water is present at high concentration in the mobile phase, as always happens at the beginning of the gradient slope, the formation of completely dry particles is compromised, influencing the remaining transfer towards the ion source and affecting the general sensitivity. In addition, due to surface active forces, water promotes the formation of bigger droplets that finally will generate larger particles. Since the effectiveness of the vaporization in a particle beam is directly related with the size of the solid particles [5,6], large particles slow down the solute vaporization and increase the chance of thermal decomposition.

In a capillary-scale particle beam (μPB) this picture is significantly improved. In fact, its lower flow-rate, typically around 1 μl/min, impose no restriction in the use of any solvent or buffer in the mobile phase [7] and the presence of water, minimized by the lower flow-rate, is well accepted.

In this work, we propose a LC–MS method for the determination of 18 phenolic compounds, including 15 benzoic and cinnamic phenolic acids, in red wines using a μPB interface and reversed-phase conditions for the separation of the analytes. Phenolic acids play an important role in defining the sensorial characteristics of wines and brandies, giving that 'oak wood taste' typical of long aged product and particularly appreciated by red wine estimators. Their composition and abundance in a certain wine is often related to the storage conditions and the aging process [8–10]. In real samples, the analyte concentrations may vary significantly and need to be determined at both low (μg/l) and high concentrations, requiring good linearity and extended dynamic range of the detector. Most of the analytical methods proposed for the separation and determination of phenolic acids rely on HPLC techniques with UV spectrophotometric and electrochemical detection [11,12]. The advantage of liquid chromatography–mass spectrometry interfacing in resolving complex mixture components is well known and it would be particularly appreciated at the low concentration level required for the determination of phenolic acids in wines. So far, the literature offers a few examples reporting the use of LC–MS in the determination of phenolic compounds. A thermospray (TSP) interface based method has been used for the analysis of cereal straw hydrolysates, which contains large amounts of phenolic compounds [13]. Two recent papers [14,15] show the use of electrospray for the determination of phenolic aldehydes, phenolic glucosides and a few phenolic acids in high-fiber dietary supplements and soybean root nodules.

The authors have already evaluated the use of a PB interface for the determination of phenolic acids [16], but in order to enhance signal response, a normal-phase, water-free separation was required [17]. Particle beam offers the undoubted advantage of library-searchable, structural information-rich mass spectra of the eluates, but on the other hand, it is often limited by the solvent composition of the mobile phase. As a matter of fact, the capillary-scale version of this interface allows a good detection limit with a good specificity, particularly suitable for the identification of phenolic acids at low concentration. An assay of the performance is presented for the entire set of analytes considered. The results concerning two wine samples subjected to different aging processes are also presented.

2. Experimental

2.1. Sample and standard preparation

For the evaluation of the method, 18 phenolic compounds, normally present to various extents in a red wine, were considered (Table 1). Of this group, nine were benzoic acid derivatives, six were cinnamic acid derivatives, and vanillin, syringic aldehyde and acetovanillone were chosen because of their importance in the typical red wine aroma. Standard solutions at a concentration of 10 mg/ml were prepared in methanol and diluted to the suitable concentration required for the study of the linearity range and the limit of detection.

For the extraction of phenolic compounds from the

Table 1
m/z and relative abundance of the most intense ions of the analytes

Compound	*m/z* (relative abundance,%)
Gallic acid (3,4,5-trihydroxybenzoic acid)	170(100), 153(85), 125(20)
3,4-Dihydroxybenzoic acid	137(100), 154(90), 109(40)
4-Hydroxybenzoic acid	121(100), 138(80), 93(40)
Gentisic acid (2,5-Dihydroxybenzoic acid)	136(100), 154(50), 80(45)
Vanillic acid (3-methoxy-4-hydroxybenzoic acid)	168(100), 153(90), 125(20)
Caffeic acid (3,4-dihydroxycinnamic acid)	136(100), 180(50), 89(45)
Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	198(100), 183(50), 127(25)
Vanillin (3-methoxy-4-hydroxybenzaldehyde)	152(100), 151(90), 123(20)
p-Coumaric acid (4-hydroxycinnamic acid)	164(100), 163(40), 147(40)
Syringaldehyde (3,5-dimethoxy-4-hydroxybenzaldehyde)	182(100), 181(50),
Acetovanillone (3,5-dimethoxy-4-hydroxyacetophenone)	151(100), 166(40), 147(40)
Sinapic acid (3,5-dimethoxy-4-hydroxybenzoic acid)	224(100), 180(60), 165(30)
Ferulic acid (3-methoxy-4-hydroxycinnamic acid)	194(100), 179(30), 133(25)
Veratric acid (3,4-dimethoxybenzoic acid)	182(100), 167(35),
o-Coumaric acid (2-hydroxycinnamic acid)	118(100), 146(60), 164(30)
Benzoic acid	122(100), 105(70), 77(50)
Salicylic acid (2-hydroxybenzoic acid)	120(100), 92(60), 138(40)
Cinnamic acid	147(100), 148(85), 103(60)

red wine samples a special procedure was applied. A 100-ml sample was deprived of its alcoholic content under vacuum at 35°C reducing its volume by 12–15%. Liquid–liquid extraction of the concentrated sample was performed with ethyl acetate after the addition of 20 g of ammonium sulfate and subsequent filtration. The procedure was repeated three times. Particulate was removed by rapid centrifugation. The extracted sample was dehydrated for 20 min with anhydrous sodium sulfate and evaporated, until dryness, under vacuum at 35°C. The residue was dissolved into 1-ml methanol–water (4:1, v/v). Prior to chromatographic injection, a further purification step was added with the elution through a C_{18} SPE cartridge (Supelclean LC-18, 500 mg, Supelco, Milan, Italy) followed by a 0.45-μm filtration. The cartridge was preconditioned with 5-ml ethyl acetate and 5-ml methanol–water (4:1, v/v) before use. Extracts were passed through the columns inserted into a vacuum manifold processor (Visiprep vacuum manifold, Supelco). Phenolic compounds retained on the sorbent beds were eluted with 2 ml ethyl acetate.

2.2. Liquid chromatography

Liquid chromatography was carried out with a Kontron Instrument 420 dual-pump, binary-gradient, conventional HPLC system (Kontron Instrument, Milan, Italy). Microliter flow-rates were obtained with a laboratory-made splitter that was placed between the pumping system and the injector [18]. This device allows conversion of almost conventional flow-rates (200 μl/min), generated by a conventional HPLC binary system, into μl/min flow-rates. A two-step splitting of the main stream of solvents generates a 2-μl/min mobile phase flow-rate with a splitting ratio of 100:1. The spitting device accurately reproduces at lower scale any solvent concentration gradient generated at higher flow-rate in the pumping system. A zero-volume connector linked a 60–500-nl internal loop injector to the splitter. A laboratory-made packed capillary column was used for the chromatographic separations. These columns are routinely made in our laboratory from 1/16 in. O.D.×250-μm I.D. polyether ether ketone (PEEK) tubing (Alltech Associates, Deerfield, IL, USA) and are packed with C_{18} reversed-phase 5-μm particle size purchased from Phase Sep (Queensferry, UK) (1 in.=2.54 cm). A 25-cm long column has a mean of 20 000 theoretical plates at 1-μl/min flow-rate and no appreciable loss of efficiency is observed for flow-rates up to 5 μl [19]. Acetonitrile was used as organic solvent in the mobile phase. Acetonitrile was preferred to methanol because of its lower

viscosity, a parameter that is crucial in μHPLC.

2.3. Particle beam mass spectrometry

This work was carried out with a Hewlett-Packard 59980B particle beam unit coupled with a Hewlett-Packard 5989A mass spectrometer. A laboratory-made micro-flow nebulizer replaced the original nebulizer [1,2]. This device generates a mobile phase aerosol using flow-rates as low as 1 μl/min. A 50-μm I.D.×180 μm O.D. fused-silica capillary tubing (Polymicro Technologies, Phoenix, AZ, USA) was used as the nebulizer tip and to connect the chromatographic column. The nebulizing gas was helium 5.6 purity grade (>99.9996%) and was purchased from SOL (Milan, Italy). The helium pressure needed was 70 to 90 p.s.i. to supply 0.1 l/min (1 p.s.i.=6894.76 Pa). The desolvation chamber temperature was kept at 40°C. The operating pressure values were 0.5 Torr in the desolvation chamber, 0.3 Torr in the second stage of the momentum separator and 8 to $10 \cdot 10^{-5}$ Torr in the manifold of the ion source (1 Torr=133.322 Pa). Mass spectrometer tuning and calibration were performed automatically using perfluorotributylamine (PFTBA) as a reference compound and monitoring m/z 69, 219, 502. The repeller potential was adjusted manually. The final tuning was optimized for m/z 219, which is the closest value to the most common fragments generated by the phenolic compounds. A mobile phase composed of water and acetonitrile at the same concentration was allowed into the ion source during calibration. The dwell times during selected ion monitoring (SIM) analyses were adjusted in order to obtain 0.5 cycles/s and a mean of ten acquisition samplings for each HPLC peak. A criterium based on two- and three-ion detection was used in SIM mode. The ions were selected on the basis of the intensity in the mass spectrum (Table 1), discarding the lowest m/z values. The final transfer tube, prior to the ion source, was shifted to a fully retracted position after the tuning procedure allowing a third pumping stage for a further sample enrichment.

2.4. Reagents and chemicals

Phenolic compounds (97–99% purity) were purchased from Fluka (Buchs, Switzerland). All solvents were HPLC grade from Farmitalia Carlo Erba (Milan, Italy) and were filtered and degassed before use. Trifluoroacetic acid (TFA) was purchased from Sigma (St. Louis, MO, USA). Reagent water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

3. Results and discussion

μPB interfacing was first developed to conteract the loss of sensitivity observed for every increase in the water concentration in the mobile phase. The principle 'less water — less problem' held, and the benefit was clearly evident in all reversed-phase applications in which water is used as the weakest solvent [4]. Phenolic acids are very well suited for reversed-phase HPLC and their response under EI conditions is appreciable and rich in structural information for a rapid identification. In a previous work [17], separation of phenolic acids in normal-phase partition chromatography was presented; a rather complex, quaternary system was required for the elution of the analytes. Since a simpler but water-rich reversed-phase separation often fails when applied to PB-MS, the normal-phase partition was coupled to a particle beam for the determination of phenolic acids [16].

In this work we demonstrate that better results for the determination of phenolic acids, in terms of reliability and sensitivity, can be obtained under reversed-phase conditions using a μPB interface. The validation of the method was based on the evaluation of chromatographic separation, instrument detection limits in the SIM mode, sensitivity and response linearity. The method was finally applied to the determination of the selected analytes in two wine samples.

The chromatographic separation was performed under reversed-phase conditions, using a 250-μm I.D. packed capillary column at a flow-rate of 1 μl/min. This type of column operates in the range of flow-rates required by the μPB for an optimal nebulization of the eluate. In addition, the column is usually compatible with all conventional 1/16-in. HPLC connectors and fittings but, due to the low flow-rate, is particularly sensitive to any extra vol-

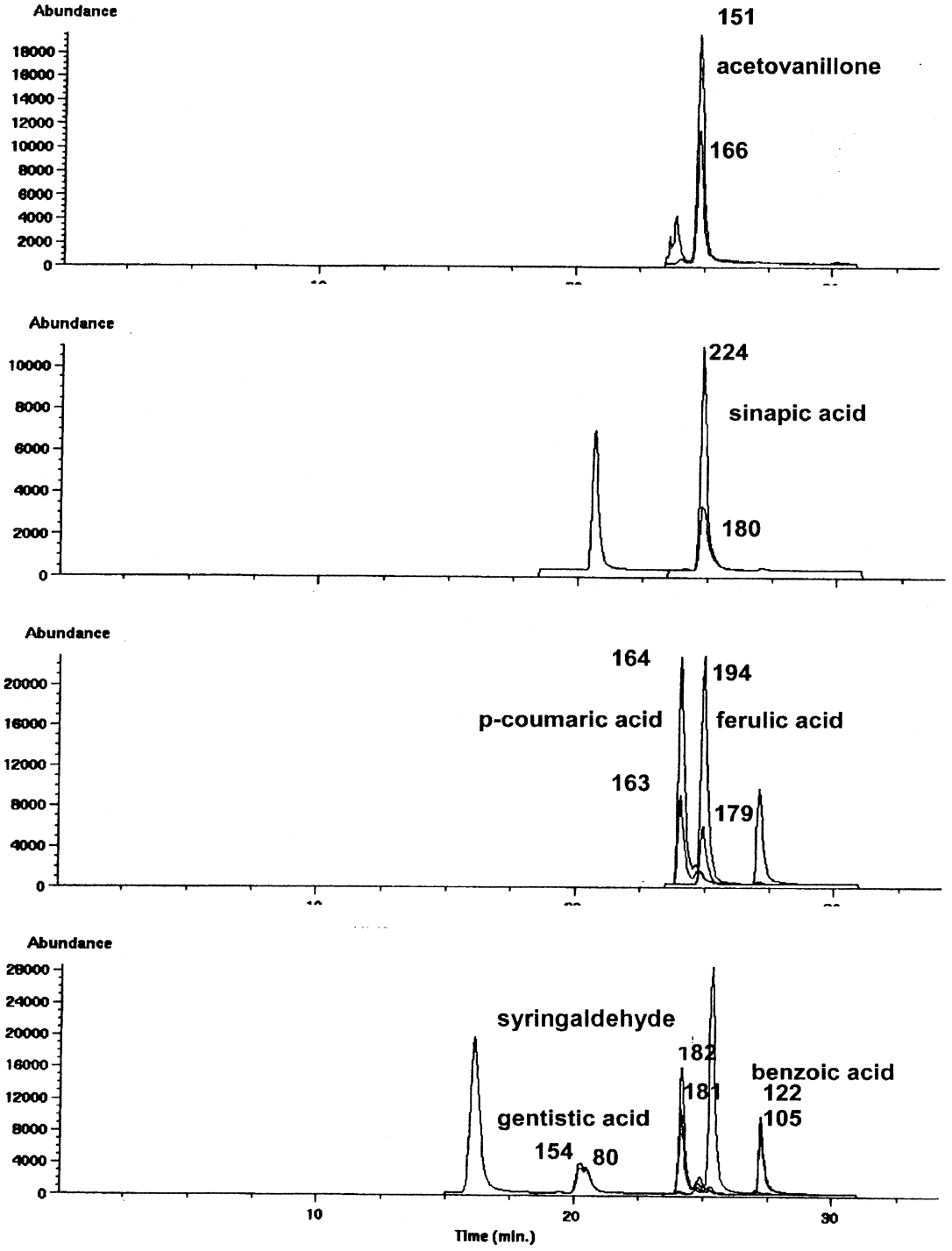

Fig. 1. Ion current profiles relative to a 0.5-μl injection of 100 ng of phenolic compounds. Chromatographic conditions: capillary C_{18} column; mobile phase, water–acetonitrile; gradient, from 100 to 20% water in 40 min; flow-rate 1 μl/min.

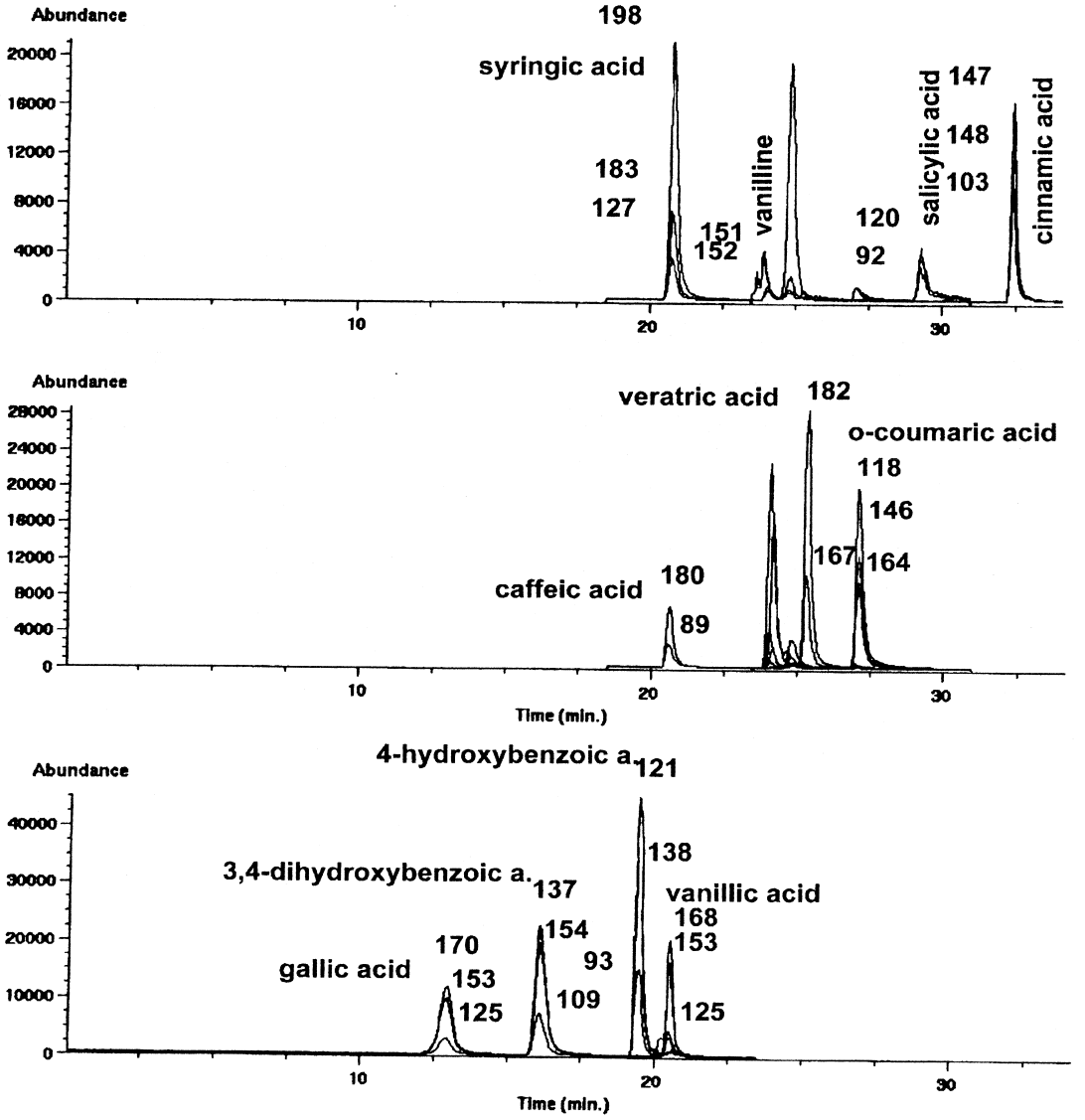

Fig. 1. (*continued*)

ume in the connecting lines. The injection volume is usually included between 60 and 500 nl and, for extremely diluted samples, can be increased up to 50-μl using special large injection volume conditions [20,21]. Although methanol offers the highest sensitivity [2], acetonitrile has the lowest viscosity among typical HPLC solvents and allows the use of longer, more efficient column at a reasonable pressure. The mobile phase was composed of water and acetonitrile. In order to suppress the acidic dissociation of the analytes, trifluoroacetic acid was added at a concentration of 0.05% in water and 0.025% in acetonitrile. The separation was achieved under gradient conditions varying the water–acetonitrile ratio from 100:0 to 20:80 over 40 min.

A preliminary test gave a good response for the phenolic compound under EI conditions. The best results were obtained with a source temperature of 230°C. Mass spectra were collected via particle beam in flow injection analysis (FIA) conditions. Full spectra were recorded as a base for generating the most convenient SIM program. For an error-free

Table 2
Retention times relative to the separation reported in Fig. 1

Compound	Retention time (min)
Gallic acid	12.9
3,4-Dihydroxybenzoic acid	16.1
4-Hydroxybenzoic acid	19.5
Gentisic acid	20.4
Vanillic acid	20.5
Caffeic acid	20.6
Syringic acid	20.8
Vanillin	23.9
p-Coumaric acid	24.0
Syringaldehyde	24.1
Acetovanillone	24.8
Sinapic acid	24.8
Ferulic acid	24.9
Veratric acid	25.3
o-Coumaric acid	27.1
Benzoic acid	27.2
Salicylic acid	29.3
Cinnamic acid	32.4

targeting of the analytes in real samples, three or at least two characteristic ions were selected for each compound. High-mass ions were privileged respect to low-mass ions (m/z <80). Low-mass ions were discarded if their relative abundance was found lower than 50%. Syringic aldehyde and veratric acid, which lack a third abundant fragment, were detected using only two ions. The intense m/z 136 ion signal, shared by the gentisic and caffeic acid mass spectra, was discarded because the column coeluted the two compounds. The corresponding data are reported in Table 1. The chromatographic run was subdivided into three time programs and each program was filled with the ions relative to the analytes eluted inside a specific time window. In this way, the dwell times increased and sensitivity was maximized. Program switchings were positioned 18.5 and at 31 min after the start. When required, the switching time was regulated manually. The ion profiles relative to the injection of a 100-ng mixture of the analytes, detected in SIM mode as above described, are reported in Fig. 1. In this case, a 200-ng/μl methanolic

Table 3
SIM instrument detection limits (IDLs) relative to phenolic compounds by monitoring 2 or 3 ions

Compound	IDL (ng)	
	(3 ions)	(2 ions)
Gallic acid	8.00	5.00
3,4-Dihydroxybenzoic acid	2.50	1.25
4-Hydroxybenzoic acid	0.80	0.30
Gentisic acid	–	5.00
Vanillic acid	1.25	0.50
Caffeic acid	–	2.50
Syringic acid	2.50	1.25
Vanillin	1.25	0.50
p-Coumaric acid	0.80	0.80
Syringaldehyde	–	0.50
Acetovanillone	0.80	0.60
Sinapic acid	5.00	2.50
Ferulic acid	2.50	2.50
Veratric acid	–	0.80
o-Coumaric acid	1.25	0.50
Benzoic acid	12.50	10.00
Salicylic acid	50.00	40.00
Cinnamic acid	0.60	0.50

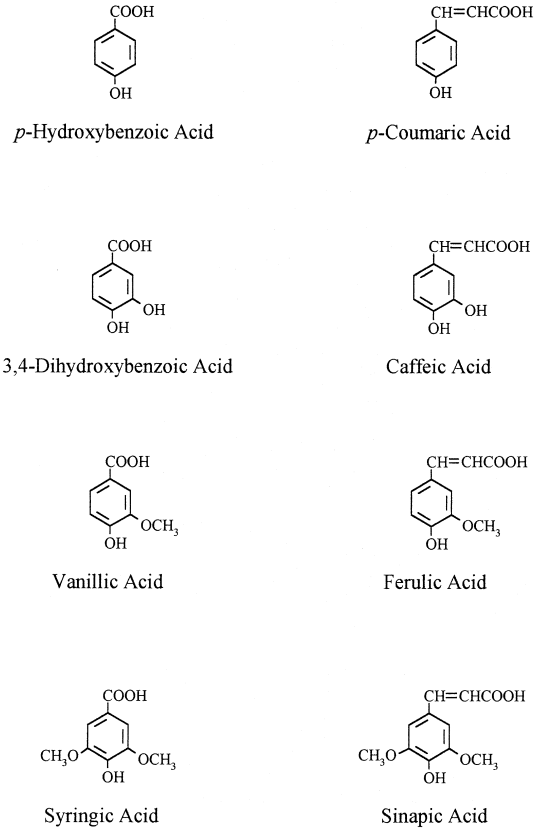

Fig. 2. Chemical structures of phenolic acids selected for linearity studies: benzoic acid derivatives (left), cinnamic acid derivatives (right).

solution of the analytes was injected via a 500-nl internal loop. The mobile phase, initially composed of 100% water, quickly replaced the sample methanol and helped solute focusing at the head of the column [20]. As result of the separation, four groups of phenolic compounds were partially coeluted from the column (Table 2) showing retention time differences shorter than a minute: group 1, gentisic acid, vanillic acid, caffeic acid, syringic acid; group 2, vanillin, *p*-coumaric acid, syringic aldehyde; group 3, acetovanillone, sinapic acid, ferulic acid; group 4, *o*-coumaric acid, benzoic acid. Although a partial coelution was observed, the components of each group shares no significant similarity in their the mass spectrum and could be well separated and identified by the mass spectrometer.

Instrument detection limits (IDLs) were obtained in the SIM mode and through the column in solvent gradient conditions. This procedure reduces the chance of presenting too optimistic data that are often far from the effective ability of detecting analytes in real-world samples. Diluted solutions of the standard mixture were injected and the signal-to-noise ratio of each ion profile carefully evaluated. IDLs were assigned when the least intense ion signal, relative to a specific phenolic compound, reached a signal-to-noise ratio of 5:1. A two-ion detection was also attempted for all the analytes considered. The slight loss in specificity was compensated by the higher signal response of the remaining ions which lowered the IDLs by approximately another 50%. The results, reported in Table 3, are significantly spread out though, from 0.3-ng for 4-hydroxybenzoic acid up to 40-ng for salicylic acid in the two-ion detection mode. The worst results were obtained for salicylic acid, benzoic acid and gallic

Table 4
Linear and quadratic regression equations relative to the concentration calibration experiments

Compound	Linear regression $y = A + Bx$ (σA and σB)	r	Quadratic regression $y = A' + B'x + C'x^2$	r^2
Vanillic acid	$A = -284.511$ (184.703) $B = 15.656$ (1.565)	0.98060	$A' = -31.069$ $B' = 3.289$ $C' = 0.049$	0.99972
3,4-Dihydroxybenzoic acid	$A = -407.220$ (283.449) $B = 8.168$ (1.020)	0.97018	$A' = 17.189$ $B' = -0.105$ $C' = 0.014$	0.99981
Syringic acid	$A = -618.259$ (334.029) $B = 14.723$ (1.202)	0.98693	$A' = -121.045$ $B' = 5.031$ $C' = 0.016$	0.99962
4-Hydroxybenzoic acid	$A = -476.495$ (330.206) $B = 39.681$ (4.754)	0.97247	$A' = 17.314$ $B' = 1.128$ $C' = 0.257$	0.99993
Ferulic acid	$A = -511.750$ (165.084) $B = 9.275$ (0.306)	0.99783	$A' = -295.458$ $B' = 7.236$ $C' = 0.002$	0.99841
Caffeic acid	$A = -453.946$ (172.447) $B = 8.100$ (0.320)	0.99689	$A' = -180.040$ $B' = 5.517$ $C' = 0.002$	0.99957
Sinapic acid	$A = -389.415$ (132.078) $B = 6.463$ (0.245)	0.99714	$A' = -199.438$ $B' = 4.672$ $C' = 0.001$	0.99865
p-Coumaric acid	$A = -195.518$ (142.458) $B = 13.259$ (1.207)	0.98383	$A' = 0.169$ $B' = 3.710$ $C' = 0.038$	0.99983

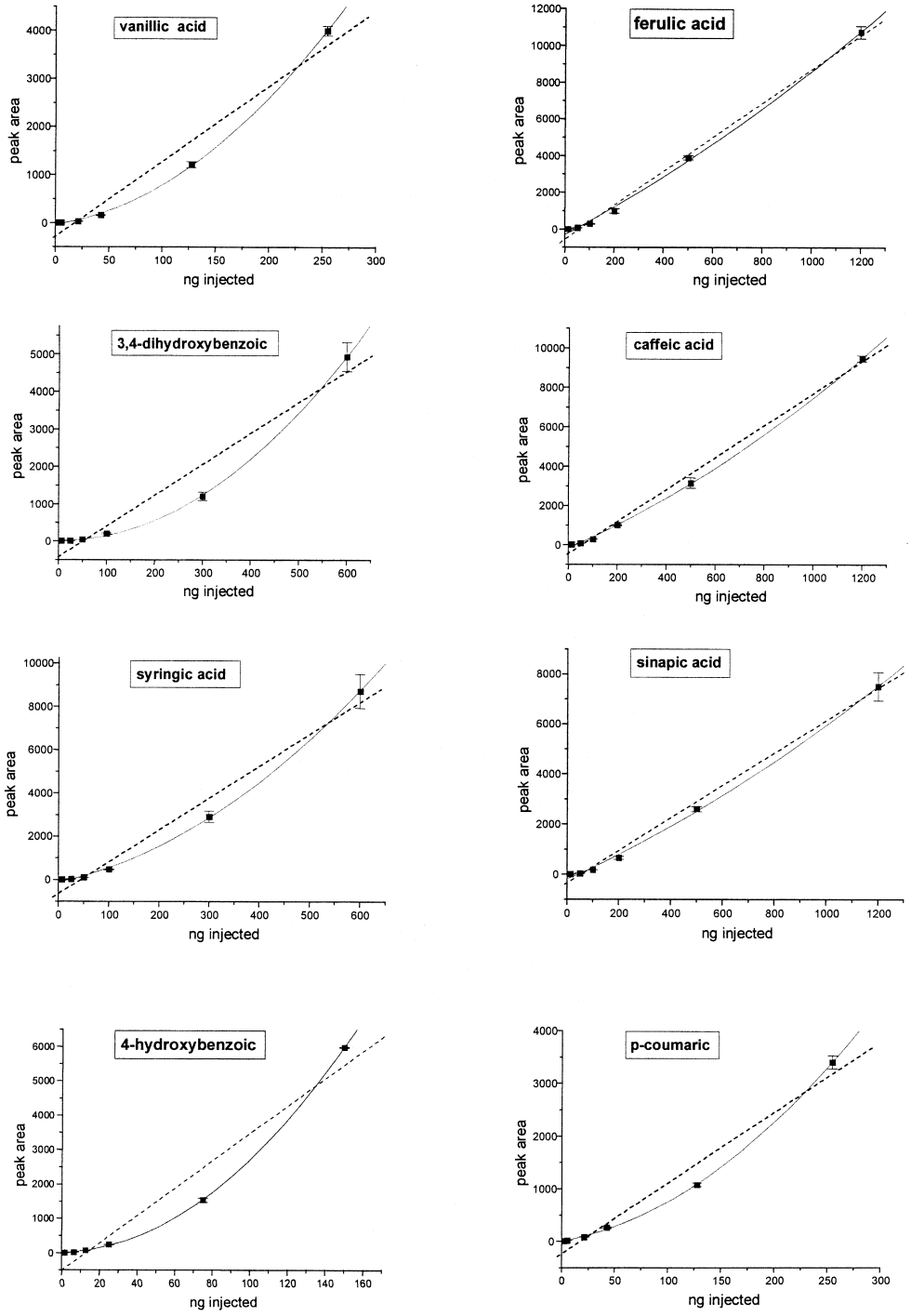

Fig. 3. Calibration graphs for selected phenolic acids. Linear and quadratic regression are reported (see text for HPLC–μPB-MS conditions).

Table 5
Intra-day reproducibility of the proposed method for the compounds selected in the concentration calibration experiment

Compound	ng	RSD (%)[a]
3,4-Dihydroxybenzoic acid	50	6.3
4-Hydroxybenzoic acid	12.5	5.2
Vanillic acid	21.25	10.9
Caffeic acid	100	2.2
Syringic acid	50	7.3
p-Coumaric acid	21.25	8.7
Sinapic acid	100	2.9
Ferulic acid	100	9.5

[a] Average of five replicates.

acid, all belonging to the benzoic acid group, but this consideration cannot be generalized since other terms of this group performed very well.

For the evaluation of the response linearity and the reproducibility of the LC–MS data, two groups of analytes were considered from the benzoic and cinnamic acid derivatives. From each group four analytes were chosen on the basis of their importance in the composition of wine and their response under LC–μPB-MS conditions. Vanillic acid, 3,4-dihydroxybenzoic acid, syringic acid and 4-hydroxybenzoic acid belonged to the benzoic acid group; ferulic acid, caffeic acid, sinapic acid and *p*-coumaric acid belonged to the cinnamic acid group. Their structures are shown in Fig. 2. Calibration experiments were performed over a wide range of concentrations, starting from five times the detection limit and then up by two orders of magnitude. The ranges were equally wide but specific for each compound since each detection limit was different and characteristic. The experiments were carried out in FIA using eluent A and B in equal proportions. The flow-rate was set at 2 μl/min and the mass spectral acquisition was carried out in the SIM mode. The standard solutions were prepared in methanol and injected using a 60-nl internal loop. Linear and quadratic regression equations and standard deviation data were calculated on the basis of five replicates for each concentration and are reported in Table 4. A visual overlook of the linear and quadratic fit is given in Fig. 3. The results obtained for the two groups of compounds are clearly different, with a better linearity for the cinnamic-related compounds. It can be assumed that because of their higher polarity, the benzoic derivatives present a higher affinity to water that, at lower concentrations, delays the desolvation of the droplets and affects, to a certain extent, the transport efficiency of the interface. The direct consequence in terms of performance is a more limited range of linearity with respect to that of the cinnamic derivatives which are slightly less polar. This effect is quantified by the linear regression fit value *r* which is also reported in the figure. The reproducibility was extrapolated from the concentration calibration data set and is reported for each compound at a single concentration level (Table

Table 6
Concentration of phenolic compounds in red wine samples obtained from Pinot Nero using two aging processes

No.	Compound	Barrique (μg/ml)	Demijohn (μg/ml)
1	Gallic acid	2282.0	5244.6
2	3,4-Dihydroxybenzoic acid	56.4	124.6
3	4-Hydroxybenzoic acid	–	11.4
4	Gentisic acid	76.8	66.8
5	Vanillic acid	148.0	172.2
6	Caffeic acid	1631.0	2903.6
7	Syringic acid	112.4	110.6
8	Vanillin	33.6	–
9	*p*-Coumaric acid	173.0	261.6
10	Syringaldehyde	–	1.2
11	Acetovanillone	13.0	10.8
12	Sinapic acid	2.4	1.9
13	Ferulic acid	32.2	44.2
14	Veratric acid	–	–
15	*o*-Coumaric acid	–	0.9
16	Benzoic acid	–	–
17	Salicylic acid	267.2	295.2
18	Cinnamic acid	3.2	2.8

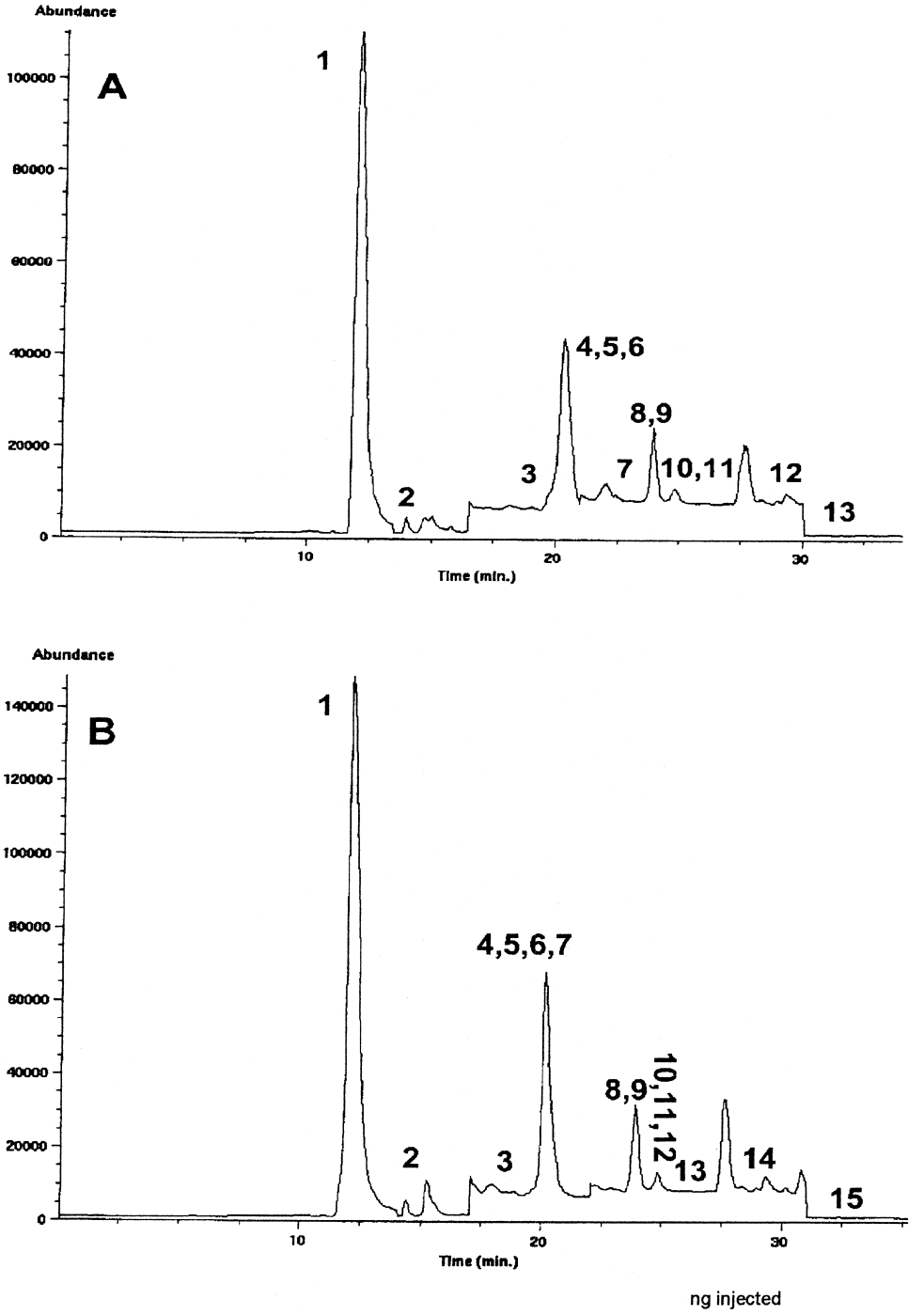

Fig. 4. On-line HPLC–μPB-MS analysis (0.5-μl volume injected) of (A) barrique and (B) demijohn aged Pinot nero wines (see Table 4 for concentration results).

5). The results are resonably good for a LC–MS method and often below 10% RSD.

The sensitivity, expressed as the slope of the linear regression equation, was higher for the 4-hydroxybenzoic acid with a value of 39.7 area counts/ng and lower for the sinapic acid where only a value of 6.5 area counts/ng was reported. For all the other compounds, intermediate values were observed.

Since the clean-up step performed by solid-phase extraction (SPE) can be considered critical for the recovery of the analytes in the sample treatment applied, the recovery of the phenolic compounds submitting a solution of a standard mixture containing the analytes to the SPE procedure was verified. A solution containing the eight analytes chosen as previously discussed was prepared at the concentration level found in the samples. The SPE step was replicated on four aliquots of the standard mixture, following the same procedure as for samples. Mean recoveries ($n=3$) were calculated by comparison of the peak area values with those obtaining by direct injection of the standard mixture. Extraction yields ranged between 90 and 95% for the benzoic derivatives and 92–102% for cinnamic acids (average RSD 5%).

The method developed was applied to the analysis of two red wine samples. The wine sample was a Pinot Nero, a famous brand name from northern Italy. The samples differed in that the aging process was carried out in a barrique in the case of the sample A and in a demijohn in the case of the sample B. Sample quantitation was obtained using an external standard calibration. A 20 ng/μl standard solution of the analytes was injected before and after the samples. The peak intensities obtained for each analyte were averaged. The identification was based on the characteristic ion profile of each analyte and the concentration was determined comparing standard and sample peak area of the most intense and characteristic ion. The results are reported in Table 6 and the relative reconstructed ion chromatograms are shown in Fig. 4. The two samples show several similarities in terms of phenolic compound composition except for gallic, 3,4-dihydroxybenzoic and caffeic acid that were more abundant in sample B. Smaller differences can be also highlighted as reported in Table 6.

4. Conclusions

A capillary-scale particle beam interface (μPB-MS) combines the typical electron ionization specificity with the high sensitivity required by a modern LC–MS interface. The reduced influence of water in the overall interfacing process, often responsible for the inadequate performance of a conventional PB interface, allows more freedom in the use of the most suitable HPLC technique. In particular, reversed-phase HPLC can be used for the separation of the analytes. The μPB interface has proven to be particularly suitable for the analysis of phenolic compounds, which gave highly informative mass spectra and intense LC–MS signals. The method was found to be sensitive enough for the determination of several phenolic compounds in red wines and for characterizing different aging processes.

References

[1] A. Cappiello, F. Bruner, Anal. Chem. 65 (1993) 1281.
[2] A. Cappiello, G. Famiglini, F. Bruner, Anal. Chem. 66 (1994) 1416.
[3] A. Cappiello, Mass Spectrom. Rev. 15 (1996) 283.
[4] A. Cappiello, G. Famiglini, J. Am. Soc. Mass Spectrom. 9 (1998) 993.
[5] A. Cappiello, G. Famiglini, A. Lombardozzi, A. Massari, G.G. Vadalà, J. Am. Soc. Mass Spectrom. 7 (1996) 753.
[6] G.G. Jones, R.E. Pauls, R.C. Willoughby, Anal. Chem. 63 (1991) 460.
[7] A. Cappiello, G. Famiglini, L. Rossi, M. Magnani, Anal. Chem. 69 (1997) 5136.
[8] G.P. Cartoni, F. Cocciolo, L. Pontelli, E. Quartucci, J. Chromatogr. 537 (1991) 93.
[9] T. Delgado, C. Gòmez-Cordovés, Rev. Franc. Oenol. 107 (1987) 39.
[10] J.A. Jindra, J.F. Galander, Am. J. Enol. Vitic. 38 (1987) 133.
[11] R. Schwarzenbach, J. Chromatogr. 251 (1982) 339.
[12] G. Chiavari, V. Concialini, G.C. Galletti, Analyst 113 (1988) 91.
[13] G.C. Galletti, J. Eagles, F.A. Mellon, J. Sci. Food Agric. 59 (1992) 401.
[14] A.M. Gioacchini, A. Roda, G.C. Galletti, P. Bocchini, A.C. Manetta, M. Baraldini, J. Chromatogr. A 730 (1996) 31.
[15] J.F. Moran, R.V. Klucas, R.J. Grayer, J. Abian, J.B. Harborne, M. Becana, Phytochem. Anal. 9 (1998) 171.
[16] C. Bocchi, M. Careri, F. Groppi, A. Mangia, P. Manini, G. Mori, J. Chromatogr. A 753 (1996) 157.

[17] M. Careri, P. Manini, G. Mori, Anal. Proc. 32 (1995) 129.
[18] A. Berloni, A. Cappiello, G. Famiglini, P. Palma, Chromatographia 39 (1994) 279.
[19] A. Cappiello, P. Palma, F. Mangani, Chromatographia 32 (1991) 389.
[20] M. Attaran Rezai, G. Famiglini, A. Cappiello, J. Chromatogr. A 742 (1996) 69.
[21] A. Cappiello, G. Famiglini, A. Berloni, J. Chromatogr. A 768 (1997) 215.