Talanta 82 (2010) 548–554

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Advantages of using a modified orthogonal sampling configuration originally designed for LC–ESI-MS to couple CE and MS for the determination of antioxidant phenolic compounds found in virgin olive oil

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ARTICLE INFO

Article history:
Received 2 November 2009
Received in revised form 19 April 2010
Accepted 10 May 2010
Available online 16 May 2010

Keywords:
Antioxidants
CE–ESI-MS
Electrospray source
Orthogonal coupling
Phenolic acids

ABSTRACT

A ThermoFinnigan sheath liquid flow capillary electrophoresis–mass spectrometry system designed for coupling via a co-axial interface was coupled through an adapted via an alternative, commercially available interface for orthogonal sampling. The affordable, reversible structural alterations made in the commercial LC–MS interface resulted in improved analytical performance.

The results of a conventional capillary electrophoresis (CE) method using a commercial co-axial source to determine antioxidant phenolic acids present in virgin olive oil, were compared with those obtained by using a modified orthogonal sampling position. In both cases, separations were done using a 10 mM ammonium acetate/ammonium hydroxide buffer solution at pH 10.0 and a constant applied voltage of 25 kV. The operating variables for the mass spectrometry interface were re-optimized for the modified orthogonal orientation. This allowed the sheath liquid, sheath gas flow rates and capillary voltage to be lowered with respect to the co-axial coupling configuration. In addition, the orthogonal sampling position provided a higher selectivity by effect of ion sampling excluding larger droplets—with an increased momentum along the axis—which were drained through the sink at the bottom of the ion source. Also, the new configuration facilitated sample ionization, improved electrospray stability and led to stronger signals as a result.

The new system was validated in terms of precision (repeatability), linearity, and limits of detection and quantification. A comparison of the validation data with the results previously obtained by using a commercial co-axial configuration revealed the adapted orthogonal sampling position to provide better repeatability in both migration times and relative peak areas (<1% and 7% respectively with $n = 15$ replicates), a good linear range (with levels in the microgram-per-litre region) and lower limits of detection—especially for the compounds detected with the lowest sensitivity when co-axial ESI was used, as HFA, GEN, FER and VAN finding LOD among 24–3.0 $\mu g\,L^{-1}$ respectively.

1. Introduction

Capillary electrophoresis–mass spectrometry (CE–MS) and tandem MS/MS, which combine the high efficiency and resolution of CE with the intrinsically high selectivity and sensitivity of MS, provide a highly attractive method for analytical determinations [1]. Ever since the initial trials of the mid 1980s and its formal presentation in 1987 [2], the CE technique has been coupled on-line with various MS systems and ionization interfaces available to developers. Worth special note among the MS systems used in this context are those based on magnetic sectors [3], quadrupoles (Q), ion traps (IT) [4], time of flight (TOF) [5,6], and Fourier transform ion cyclotron resonance (FTICR) equipment [7–12], as well as the off-line combination of CE with matrix-assisted laser desorption/ionization (MALDI) following deposition of the eluted sample on a matrix [13–15]. Some authors have used CE–MS with specific ionization systems such as continuous flow fast atom bombardment [16,17], laser vaporization ionization using UV laser [18] or sonic spray ionization [19].

As regards ionization interfaces, electrospray ionization (ESI) has been deemed a highly efficient choice for coupling CE with MS [20,21]. In fact, ESI allows the detection of multiple chargeable species of a high molecular mass and molecules can be directly transferred from the separation capillary to the mass spectrometer via the interface [22]. ESI is also a soft ionization method inasmuch as it allows the formation of gas phase ions via a gentle process that enables the sensitive analysis of non-volatile and thermolabile

Abbreviations: FER, ferulic acid; GEN, gentisic acid; HFA, hydroxyphenyl acetic acid; MT, migration time; o-, *m*-, *p*-COU, *o*-, *m*-, *p*-coumaric acid; RPA, relative peak area; S/N, signal/noise ratio; VAN, vanillic acid.

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doi:10.1016/j.talanta.2010.05.005

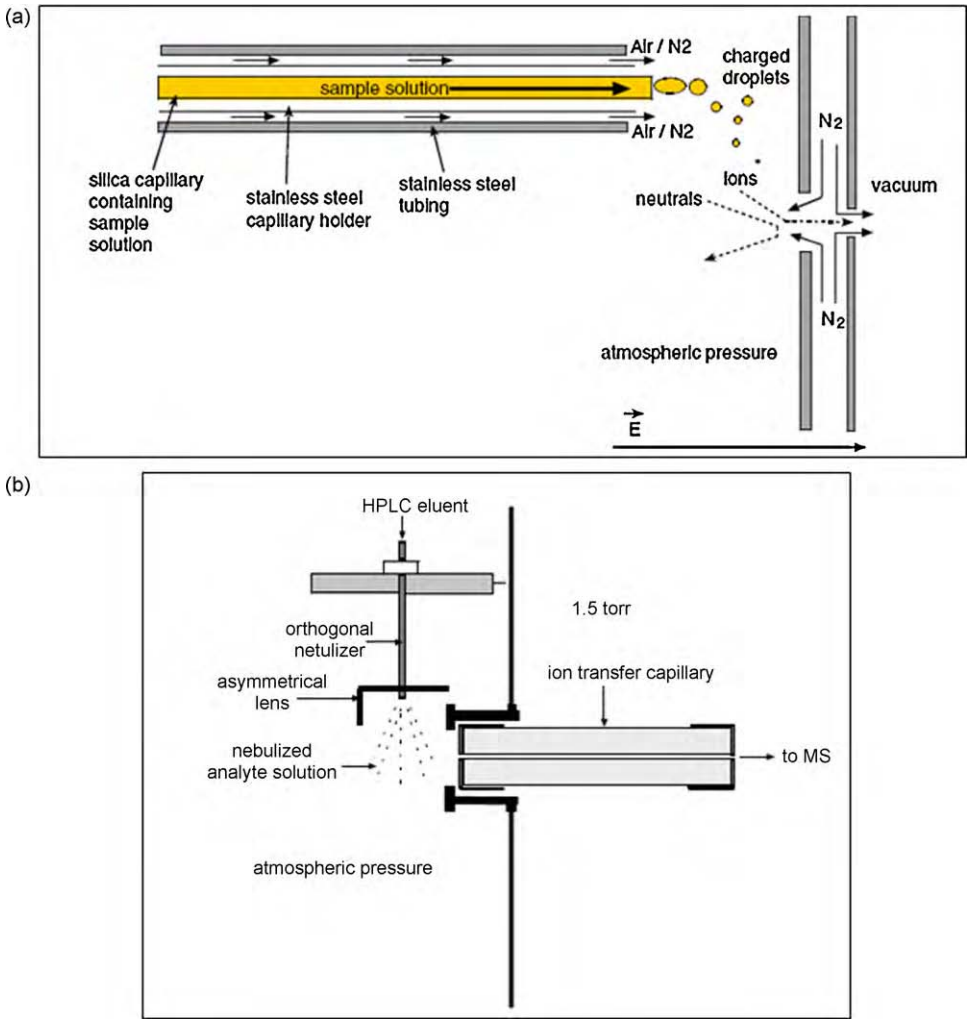


Fig. 1. Schematic depiction of (a) the ion spray configuration developed by Bruins in 1987 and (b) the orthogonal ESI source configuration for LC developed by Agilent Technologies (adapted from [30], Figs. 5 and 7, with permission of Elsevier).

compounds. As a result, the use of ESI sources in mass spectrometry (MS) has greatly facilitated the study of large biomolecules, as well as pharmaceutical drugs and their metabolites. In fact, ESI sources have evolved in parallel with proteomics and drug discovery research [23].

ESI interfaces are essentially of either of two types, namely liquid-supported systems [24] and non-liquid-supported systems also referred as "sheathless flow interfaces" [25,26].

The liquid sheath flow interface was originally developed by Smith et al. [27] and is the more common at present. Thus, it has been used in about 77% of cases to connect CE with ESI-MS by virtue of its providing electrical contact and a constant flow in addition to increased reproducibility and robustness relative to existing liquid junction interfaces [28,29].

Ever since the enormous bioanalytical potential of ESI was recognized in the late 1980s, researchers have strived to exploit its capabilities by modifying the source geometry in order to expand the ranges of flow rates and source fragmentation, as well as to improve sensitivity, efficiency and practicality [30]. The 1998 and 2003 review papers by Niessen [31,32] provide a good starting point for any readers interested in following the evolution of ESI sources. For example, with flow rates in the range 0.05–3 mL min^{-1} (*i.e.* the high region), sensitivity can be an issue by effect of the decreased ionization efficiency resulting from the large size of the droplets formed. One solution to this problem can do simply by re-orienting the sprayer relative to the interface so that the fine droplets from the outside of the spray plume can enter the sampling inlet, while most large droplets are directed away from the entrance [33]. In 1987, Bruins [34] found the spraying process to be less markedly dependent on the sprayer position relative to the orifice than without nebulizer assistance, and also that better sensitivity was obtained if the sprayer was pointed off axis instead of directly at the orifice (see Fig. 1a). The reasoning behind the off axis geometry was that, by sampling the periphery of the spray, finer droplets entered the mass spectrometer while the larger droplets struck the curtain plate. This led to improved performance by effect of finer droplets being easier to desolvate. Later, electrospray stability has been improved and contamination of the source minimized by switching from the off axis sprayer geometry to an orthogonal sampling position (Fig. 1b). A number of commercial MS instruments now sample orthogonally from the spray plume for many applications.

In previous work, we used an LCQ DECA XP Plus spectrometer from Thermo Finnigan in the co-axial sampling mode in combination with capillary electrophoresis, and encountered the above-described problems for this specific sampling configuration (*e.g.* poor selectivity, high instrumental noise). In this currently published paper [35], we used co-axial sampling in CE–MS equipment to develop an analytical method for the determination of antioxidant phenolic acids in virgin olive oil by CE–ESI-MS. In the

present work, we introduced an innovator, simple and reversible structural modification in the original design (*viz.* the orthogonal sampling position) in order to improve the analytical results. In addition, we assessed the advantages of the new configuration in terms of stability, reproducibility, linearity and sensitivity over the original design. The target compounds used for this purpose were phenolic acids, which are currently receiving much attention as essential nutritional ingredients of olive oil that are partly responsible for its colour, astringency, bitterness and flavour [36–38] playing a central role in human nutrition as protective agents against various conditions including cardiovascular disease [39–41].

2. Materials and methods

2.1. Reagents

All antioxidants studied (Table 1), both benzoic and cinnamic acid derivatives, were purchased from Sigma–Aldrich (St. Louis, MO). Ammonium acetate, ammonium hydroxide and acetic acid, all analytical-grade, were obtained from Panreac (Barcelona, Spain).

Ultrapure water from a Milli-Q apparatus (Millipore, Milford, MA) was used to prepare all solutions including the background electrolyte. A 1 M solution of sodium hydroxide purchased from Panreac (Barcelona, Spain) was used to rinse the capillary.

Nitrogen gas for the LCQ™ and helium damping gas for the ion trap were both supplied by Air Liquide (Madrid, Spain).

2.2. Solutions and samples

2.2.1. Standard solutions

Standard stock solutions of the analytes were prepared by dissolving an appropriate amount of each pure substance in 25 mL of analytical-grade ethanol to obtain a final concentration of 1000 mg L^{-1}. The resulting solutions were stored at 5 °C in topaz glasses.

Working standard solutions at a 2 mg L^{-1} concentration were prepared on a daily basis by diluting appropriate aliquots of the previous standard stock solutions in Milli-Q water.

When some variable related to the CE–MS interface was to be modified, appropriate *tuning standard solutions* containing a 20 mg L^{-1} concentration of each antioxidant were prepared and diluted with running buffer.

2.2.2. Background electrolyte

Phenolic acids were separated in a background electrolyte (BGE) consisting of a 10 mM ammonium acetate/ammonium hydroxide buffer solution at pH 10.0 that was prepared by weighing the required amount of ammonium acetate and adjusting its pH with a few drops of ammonium hydroxide. The running buffer was prepared on a daily basis because stored solutions resulted in unstable CE and ESI currents, and hence in irreproducible migration times (MTs) owing to the high volatility of ammonia, among other factors.

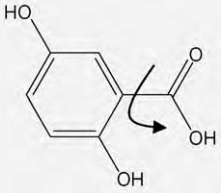
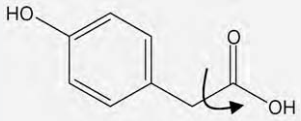
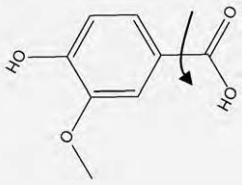
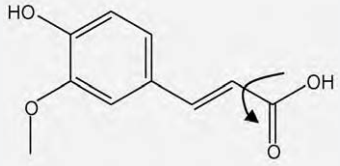
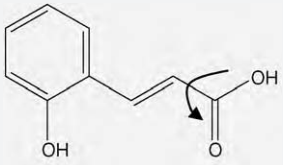
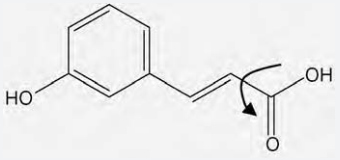
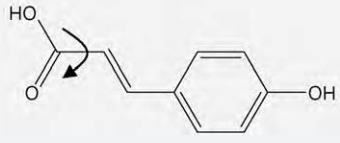
All solutions and buffers were degassed by sonication for 5 min before use in order to avoid changes during ionization and ensure acceptable reproducibility.

pH measurements were made with a Crison model 2002 pH meter furnished with a combined glass electrode.

2.2.3. Sheath liquid

The sheath liquid used for detection in the ESI-negative mode was a solution consisting of 75% 2-propanol and 5 mM running buffer (ammonium acetate/ammonium hydroxide, pH 10.0). This solution was also freshly prepared each day and degassed by sonication for 5 min prior to use in order to ensure proper, reproducible ionization.

Table 1
Chemical structures and mass spectrometry (ESI-MS) data for the studied phenolic acids.

Compounds	Structures	$[M-H]^-$ (m/z)
Benzoic acid derivates		
Gentisic acid (GEN)		153
Hydroxyphenyl acetic acid (HFA)		151
Vanillic acid (VAN)		167
Cinnamic acid derivates		
Ferulic acid (FER)		193
o-Coumaric acid (o-COU)		163
m-Coumaric acid (m-COU)		163
p-Coumaric acid (p-COU)		163

2.3. Instrumentation

A P/ACE System MDQ capillary electrophoresis instrument equipped with a diode-array detection (DAD) system governed by dedicated capillary electrophoresis software, all from Beckman (Fullerton, CA, USA), was used for measurements.

The detector was an LCQ DECA XP Plus mass spectrometer from ThermoFinnigan (San Jose, CA) equipped with a tri-co-axial pneumatically assisted electrospray ionization (ESI) source specially

designed for coupling CE to MS and equipped with an ion trap (IT) as analyser. An orthogonal interface designed for coupling LC to MS was easily adapted via reversible modifications for orthogonal sampling in the CE–MS equipment, in order to assess the ensuing improvements in analytical parameters.

2.4. CE separation

Separations were done on 80 cm fused-silica capillaries (75 μm i.d., 375 μm o.d.), using a 10 mM ammonium acetate/ammonium hydroxide buffer solution at pH 10.0 that was previously degassed by sonication as running electrolyte. Prior to first use, the untreated capillary was conditioned by consecutive flushing with 1.0 M NaOH at 20 psi for 10 min and water at 20 psi for 10 min, followed by conditioning with BGE at 20 psi for 5 min. Then, samples were injected in the hydrodynamic mode (15 s at 1.0 psi) and separation effected by applying a constant voltage of 25 kV (a voltage ramp of 1.30 kV s^{-1}), which resulted in a generated current of approximately 30 μA. The electrophoretic medium was kept at 25 °C throughout.

Different electrolyte vials were used for rinsing and separation in order to maintain a constant electrolyte level on the anode side. Each set of separation vials was changed after 4 separation runs.

Prior to overnight storage, the capillary was flushed with 1.0 M NaOH and water for consecutive periods of 10 min, followed by drying with air for 3 min.

The quantitative results for validation were calculated from relative peak areas (RPA, analyte peak area/internal standard peak area) in all cases.

2.5. ESI-MS detection

The sheath liquid was a solution consisting of 75% 2-propanol and 5 mM running buffer that was passed at a flow rate of 2 μL min^{-1}. The sheath gas flow rate was set at 20 on the scale of arbitrary units (a.u.), provided by the instrument software and the auxiliary gas flow rate to 5 a.u.

The heated capillary temperature was held at 200 °C and the electrospray needle voltage set at 2.00 kV. A portion about 5 cm in length of the poly-imide coating was removed from the fused-silica capillary in order to avoid dissolution by the sheath liquid and minimize contamination of the electrospray source as a result.

Distances, lengths and positions were chosen in such a way as to maximize electrospray stability. Different lengths measured with a micrometer screw were tested in combination with a distance of *ca.* 0.5 mm between the protruding portion of the CE capillary and electrospray needle. Two additional distances arising in the adapted orthogonal interface were also studied. One was the distance between the electrospray needle and heated capillary or MS inlet, which was designated *longitudinal displacement* (front–back distance, *Y*-axis) and divided into four segments (sub-positions) the longest of which was 1.7 cm. The other was the distance between the electrospray needle and MS detector inlet, which was designated *lateral displacement* (right–left distance, *X*-axis) and divided into three segments (sub-positions) of increasing length from A to M to F.

Mass spectrometry data were processed by using Xcalibur 1.4 software. CE–MS data were acquired throughout the *m/z* range (135–195) using the centroid mode, negative polarity and an injection time of 300 ms. Scans were done at 3 μs intervals.

The MS detector was calibrated with a new tune method involving the infusion of *tuning standard solutions* containing a 20 mg L^{-1} concentration of each of seven phenolic compounds and an internal standard, diluted in running buffer through the capillary into the detector at an injection pressure of 0.5 psi under a constant voltage of 2.0 kV.

3. Results and discussion

3.1. Instrumentation design. Coupling CE to MS via a commercial LC–MS interface adapted for orthogonal sampling

A number of currently available MS instruments use orthogonal sampling from the spray plume in many applications in order to increase electrospray stability and minimize potential contamination of the source with background electrolyte or sheath liquid [30].

The commercial mass spectrometry interface from Thermo Finnigan used here was specially developed for coupling capillary electrophoresis in the co-axial sampling position. However, we replaced it with an orthogonal interface for liquid chromatography that was subjected to simple and reversible structural modifications for the intended purpose.

Fig. 2 depicts the individual components used for the reversible transformation of the commercial LC interface with a view to its use as an adapted CE–MS interface for orthogonal sampling. The specific changes required were as follows:

(a) Using the LC/ESI source (orthogonal orientation) required adapting the LC-MS-ESI probe for use as a CE–MS–ESI probe (1) in order to maintain electrical contact, via the sheath liquid, with the capillary electrophoresis system.
(b) Using a laboratory-made metal ring (2) with an extension about 4 cm long to attach the ESI probe to the micrometer assembly (3).

As in co-axial sampling, a micrometer screw was used to adjust the distance of the protruding portion of the CE capillary to the electrospray needle.

This entailed precisely fitting the metal ring to the metallic outlet of the LC–MS interface in order to allow the simultaneous displacement of the micrometer screw and interface block, and ensure optimal operation.

3.2. Optimization of mass spectrometry interface variables

Some CE variables such as electrolyte pH and ionic strength, separation voltage and injection-related parameters, should be kept at their optimal values irrespective of the relative orientation of the source. This led us to adopt the optimum parameter values for CE–MS with a co-axial source reported elsewhere [35] to determine antioxidant phenolic acids in the modified (orthogonal) sampling position.

Table 2 summarizes the settings used in the two sampling configurations.

The geometry of an electrospray ion source is known to influence analyte desolvation, ionization, transfer and detection in a mass spectrometer [30]. This led us to re-optimize some MS parameters including the interface and electrospray conditions in order to increase electrospray stability and improve analytical parameters such as signal intensity, signal/noise ratio (S/N) and peak shape.

A previously optimized *sheath liquid* consisting of 75% 2-propanol and 5 mM running buffer (ammonium acetate/ammonium hydroxide, pH 10.0) was also used in this work. Its optimum flow rate was taken to be that minimizing dilution of the analytes in order to ensure acceptable sensitivity and spray stability. The influence of the flow rate over the range 1–5 μL min^{-1} was studied, and a value of 2 μL min^{-1} was selected as optimal on the grounds of its resulting in the best peak shape for all compounds in addition to increased peak intensities and S/N values for most relative to the results of previous work.

The influence of the *sheath gas* flow rate was studied over the range 10–40 arbitrary units (a.u.) and a value of 20 a.u. was adopted

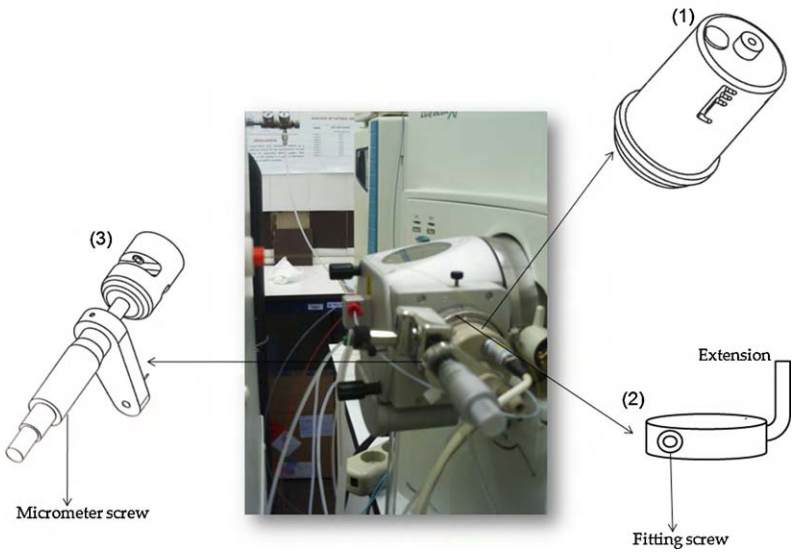


Fig. 2. Main components used for adapted orthogonal sampling with a commercial orthogonal interface for LC–ES-MS (ThermoFinnigan). (1) CE ESI probe, (2) metal ring, (3) micrometer assembly. 1 and 2 are reprinted from "*Getting started: CE/MS adapter for the Finnigan LC/ESI source*. Revision C 97000-97008". The photography shows the adapted orthogonal orientation used for separation by CE–MS.

as a compromise between peak area and S/N, and also because it sufficed to ensure efficient analyte desolvation and ionization. However, because the sheath gas flow rate was halved relative to the original source orientation (Table 2), a new variable called the *auxiliary sheath gas flow rate* was introduced. With orthogonal sampling, the auxiliary sheath gas flow is usually directed upstream in order to increase the ionization efficiency by facilitating the formation of much smaller droplets. The influence of the auxiliary sheath gas flow rate was studied over the range 5–20 a.u. and a value of 5 a.u. was selected since greater values resulted in rather broad peaks and essentially identical S/N values, so raising the flow rate at the expense of consuming more nitrogen was unwarranted.

Table 2
Comparison of the CE–MS settings used for co-axial and orthogonal sampling.

Parameter	Co-axial sampling[a]	Orthogonal sampling
BGE composition	10 mM ammonium acetate/ ammonium hydroxide buffer, pH 10.0	
Capillary dimensions	80 cm, 75 μm i.d. and 375 μm o.d.	
Separation voltage	25 kV	
Temperature	25 °C	
Injection	10 psi for 15 s	
Sheath liquid composition and flow rate	75% 2-propanol and 5 mM BGE at 3 μL min^{-1}	75% 2-propanol and 5 mM BGE at 2 μL min^{-1}
Sheath gas auxiliary flow rate	40	20/5
Spray voltage	3.75 kV	2.0 kV
Heated capillary temperature	200 °C	200 °C
Distance from CE-to electrospray needle	0 mM	0 mM
Distance from CE–MS to heated capillary	1.5 cm	3-A position
Injection time for IT	300 ms	300 ms

[a] Data reported elsewhere [35].

The effect of the *electrospray voltage* was studied over the range 1.0–3.0 kV. Above 2.0 kV, however, peaks grew wider and S/N remained essentially unchanged. On the other hand, voltages lower than 2.0 kV detracted from signal intensity. An electrospray voltage of 2.0 kV was therefore selected as optimal for all target compounds.

The effect of the *length of CE capillary protruding from the electrospray needle* was examined over the range 0–0.5 mm with the aid of a micrometer screw. The maximum signal with both co-axial and orthogonal sampling was obtained when the whole CE capillary was inserted in the ES needle (*i.e.* with no protrusion) as the likely result of instability in the formation of charged droplets being avoided.

As noted earlier, using an orthogonal orientation of the source entailed defining two additional distances (longitudinal displacement and lateral displacement). The longitudinal displacement was divided into four segments representing as many sub-positions (4.25, 8.50, 12.75 and 17.00 mm, respectively). The lateral displacement was divided into three segments defining the sub-positions A, M and F from nearest to farthest. The effect of the two distances was studied simultaneously since they were mutually dependent.

The combination of a longitudinal displacement of 12.75 mm (third position) and sub-position A (the closest) between ES needle and MS inlet was selected as the best compromise between peak area and S/N. The added difficulty of determining both optimum positions jointly, led us to determine the relative standard deviation (RSD, %) for 10 replicates. The lowest RSD was that for the previously selected distances.

Table 2 compares the optimum CE–MS parameter values with for the original, co-axial orientation and the orthogonal sampling position. As can be seen, some ESI-MS parameters such as the heated capillary temperature and injection time for ion trapping were also examined and their optimum values found to coincide with previously reported data.

Fig. 3 shows the base peak electropherogram and extracted ion electropherograms for the studied phenolic acids (the internal standard, salicylic acid, included) as obtained under optimal conditions for the ESI-MS interface. Also, the figure compares the analytical results for MTs, absolute areas and S/N values obtained with co-axial sampling [35] and orthogonal sampling.

Compound	Co-axial sampling			Orthogonal sampling		
	MT (min)	Absolute Area	S/N	MT (min)	Absolute Area	S/N
HFA	8.45	1089595	13	10.86	3348855	16
GEN	9.04	1279813	88	11.72	8175878	148
m-COU	9.14	2997667	172	11.50	10548474	107
o-COU	9.92	2794562	159	12.34	8547903	121
p-COU	10.22	3058044	166	12.80	12656389	160
FER	9.69	2815583	92	12.19	9390651	128
VAN	10.49	1587646	60	13.10	6204895	39
I.S.	10.19	12345600	61	12.11	12766265	137

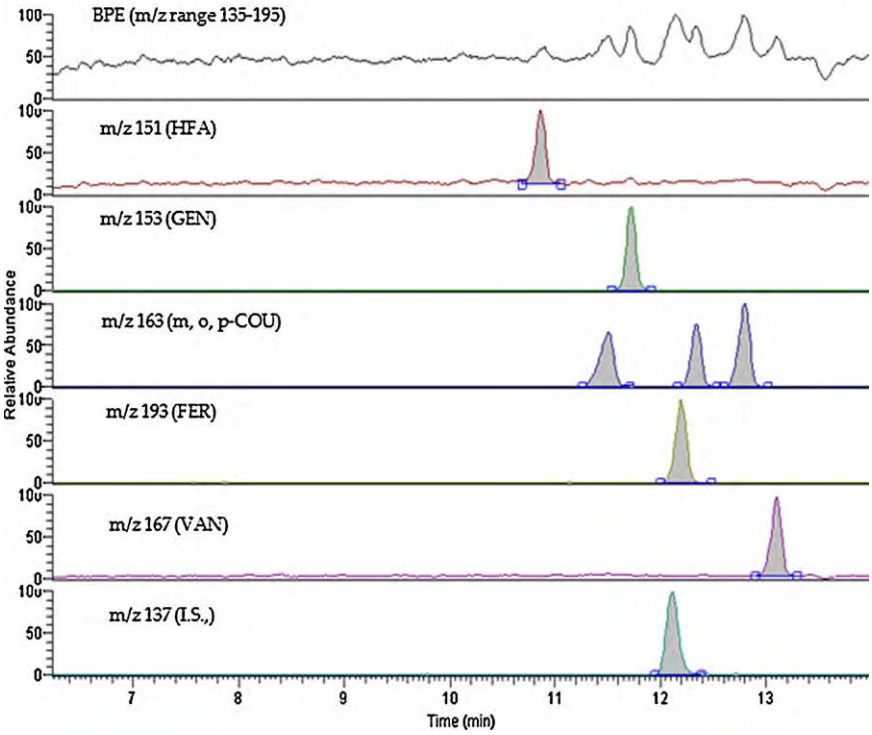


Fig. 3. Base peak electropherogram (BPE) and extracted ion electropherograms obtained during separation of the studied phenolic compounds at 2 mg L^{-1} concentration each. Comparison of the analytical parameter values obtained by using a commercial co-axial source and the adapted orthogonal sampling configuration. Conditions for co-axial and orthogonal sampling are summarized in Table 2.

As can be seen, the commercial interface provided shorter MTs by effect of its using different capillaries in different experiment for electrophoretic separation. On the other hand, orthogonal sampling resulted in much higher absolute areas and similar or higher S/N values for all compounds except m-COU, p-COU and VAN.

3.3. Validation of the orthogonal sampling results

A comparison of the validation results as regards electrospray stability, sensitivity and linearity (Table 3) between orthogonal sampling and co-axial sampling was done, in order to identify potential advantages in the former. The comparison included precision (repeatability), and limits of detection (LODs) and quantitation (LOQs). Validation was based on the average relative peak areas (RPA) obtained from triplicate injections of standard mixtures of samples containing 2 mg L^{-1} salicylic acid as internal standard.

The *precision* of CE–ESI-MS with the commercial and adapted interfaces was compared in order to identify the more reproducible configuration. The precision of both was determined in terms of repeatability, using 15 replicates of a standard solution containing a 2 mg L^{-1} concentration of each compound—the internal standard included.

Table 3
Comparison of the validation results for the studied acids as obtained with co-axial and orthogonal sampling.

Co-axial sampling[a]					Orthogonal sampling			
Acid	Repeatability (RSD, n = 15, in RPA)	LOD (μg/L)	Linear range (mg/L)	R^2	Repeatability (RSD, n = 15, in RPA)	LODs (μg/L)	Linear range (μg/L)	R^2
HFA	10.3	60.0	0.4–16.0	0.9993	7.1	24.0	80–600	0.9988
GEN	10.7	500	2.0–40.0	0.9562	5.3	4.0	20–150	0.9962
m-COU	7.2	5.5	0.05–2.0	0.9889	5.9	3.5	25–187	0.9964
o-COU	5.2	3.5	0.3–1.3	0.9852	5.6	3.5	25–187	0.9930
P-COU	8.2	6.0	0.05–2.0	0.9919	3.9	3.0	30–187	0.9946
FER	9.3	14.0	0.1–4.0	0.9979	7.2	3.0	12–90	0.9882
VAN	6.4	11.0	0.1–4.0	0.9939	6.4	5.0	40–300	0.9944

[a] Data reported elsewhere [35].

The relative standard deviation (RSD) for MTs, RPA and S/N was much lower with the adapted orthogonal interface. Thus, RSD was less than 1.0% for MTs and ranged from 4.0 to 7.0% for RPA, both being lower than the respective values obtained with co-axial sampling. Also, an RSD of 10% for S/N was obtained with co-axial sampling versus 8.0% with orthogonal sampling.

Limits of detection (LODs) were estimated by sequentially injecting increasingly low concentrations of the studied analytes until no detectable signal was obtained. *Limits of quantitation* (LOQs) were calculated by multiplying LODs by 10/3. As can be seen from Table 3, the LODs for the less sensitive compounds (HFA, GEN, FER and VAN) were significantly lower with orthogonal sampling, whereas those for the others (*e.g.* coumaric acid isomers) were similar or slightly lower than those obtained with co-axial sampling.

After LOQs were calculated, the *linearity* of the proposed method was assessed by plotting RPA for each compound against its concentration. Linear regression equations, coefficients of determination (R^2) and linearity ranges were thus calculated and compared with the previously reported results.

The data of Table 3 confirm the improved precision, sensitivity and linearity obtained with orthogonal sampling.

4. Concluding remarks

In this work, original, simple and reversible modifications allowed a commercial LC/ESI interface to be adapted for orthogonal sampling with improved analytical performance in CE. The adapted configuration was used to develop a new method for the determination of antioxidant phenolic acids present in virgin olive oil by CE–MS.

The optimum parameter values for the modified method using orthogonal sampling were compared with those reported elsewhere [35] for co-axial sampling with a commercial interface. Based on the data of Table 2, the adapted orthogonal interface requires lower sheath liquid and auxiliary sheath gas flow rates, and an also lower capillary voltage, than the commercial interface. This results in decreased consumption of sheath liquid and sheath gas in addition to improved electrospray stability.

The procedure was validated by determining its precision, limits of detection and linearity (see Table 3). The results confirmed that the adapted orthogonal position provides better repeatability in migration time (MT), relative peak area (RPA) and signal/noise ratio (S/N) for all studied compounds in addition to good linearity and lower limits of detection [especially for the analytes exhibiting the lowest sensitivity (HFA, GEN, FER and VAN)].

Acknowledgement

The authors would like to thank Ministerio de Ciencia e Innovación (Spain) for the financial support through Project CTQ2008-02126/BQU.

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