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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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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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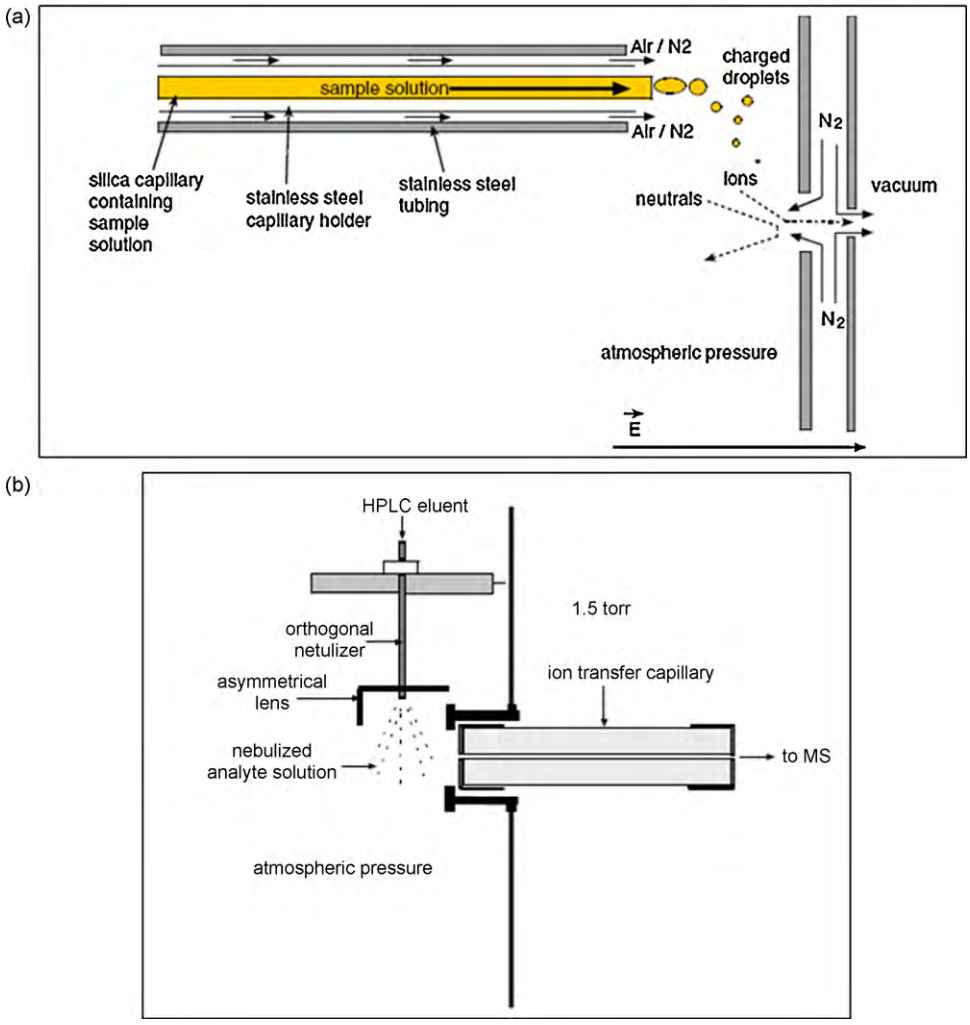

Fig. 1. Schematic depiction of (a) the ion spray configuration developed by Bruins in 1987 and (b) the orthogonal ESI source configuration for LC developed by Agilent Technologies (adapted from [30], Figs. 5 and 7, with permission of Elsevier).

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