



Natural compounds analysis using liquid and supercritical fluid chromatography hyphenated to mass spectrometry: Evaluation of a new design of atmospheric pressure ionization source

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ABSTRACT

The MS hyphenation performance of UniSpray[®], a new design of atmospheric pressure ionization source was evaluated in both SFC and RPLC modes. Sensitivity, stability, versatility and matrix effects offered by the UniSpray were assessed in positive and negative ionization modes and systematically compared to an electrospray source (ESI) using 120 natural compounds covering an extended chemical space. In a first instance, a multivariate approach was used to screen and optimize the UniSpray source settings to maximize detection sensitivity. The position of the source capillary against the fixed charged rod was highlighted as the major parameter affecting the detection sensitivity. The sensitivity improvement in UniSpray vs. ESI strongly depends on the compounds chemical class and the chromatographic mode. For a few compounds (i.e. anabasine, theanine, caproic acid, fumaric acid and protopanaxatriol), up to a 10-fold increase in sensitivity was noticed with UniSpray. The signal stability over multiple injections was also found to be equivalent between both sources with RSD values on peak intensity lower than 14% on > 100 injections, in both chromatographic modes. On complex plant extract, the matrix effects occurring from the secondary metabolites were also found to be comparable between ESI and UniSpray, at least in the positive ionization mode. However, a systematic decrease of MS signal intensity was observed in SFC mode when compounds were ionized using UniSpray in the negative ion mode.

1. Introduction

Plant secondary metabolites have been and remain a significant source of original and chemical entities. Their properties are of great interest for pharmaceutical industries and already led to the development of several drugs [1,2]. In the fields of nutrition, food supplements, functional foods and nutraceuticals, investigations are currently exploring the wide bioactivity potential of natural products on disorders that are highly influenced by the diet such as obesity, gastrointestinal health and neurodegenerative diseases [3,4]. However, the characterization of plant metabolites remains challenging, due to the rich variety of their physicochemical properties (i.e. lipophilicity, polarity, acidobasic properties, hydrogen bond capabilities, chiral center and molecular mass) and their extended endogenous concentrations range [5]. Investigations of natural compounds chemical space require sensitive, versatile and complementary techniques [6]. During the last decade, an

advent of metabolomic techniques was observed in parallel to an improvement of chromatographic techniques hyphenated to high-resolution tandem mass spectrometers (HRMS) leading to new possibilities in natural product applications. Thus, state-of-the-art ultra-high performance supercritical fluid chromatography (UHPSFC) and ultra-high performance liquid chromatography (UHPLC) coupled to a quadrupole time-of-flight tandem mass spectrometer (QqTOF-MS) have been successfully applied for the extended investigation of natural products [7,8]. Followed by adequate data treatment strategies, untargeted investigations on those platforms have led to putative dereplication and the identification of natural bioactive [9,10]. A current limitation of chromatographic techniques hyphenated with MS relies on the fact that only the molecules that could be efficiently ionized and transmitted to the MS may be detected with suitable sensitivity. Atmospheric pressure ionization (API) interfaces constitutes the most widely used sources to couple chromatographic techniques using a condensed mobile phase to

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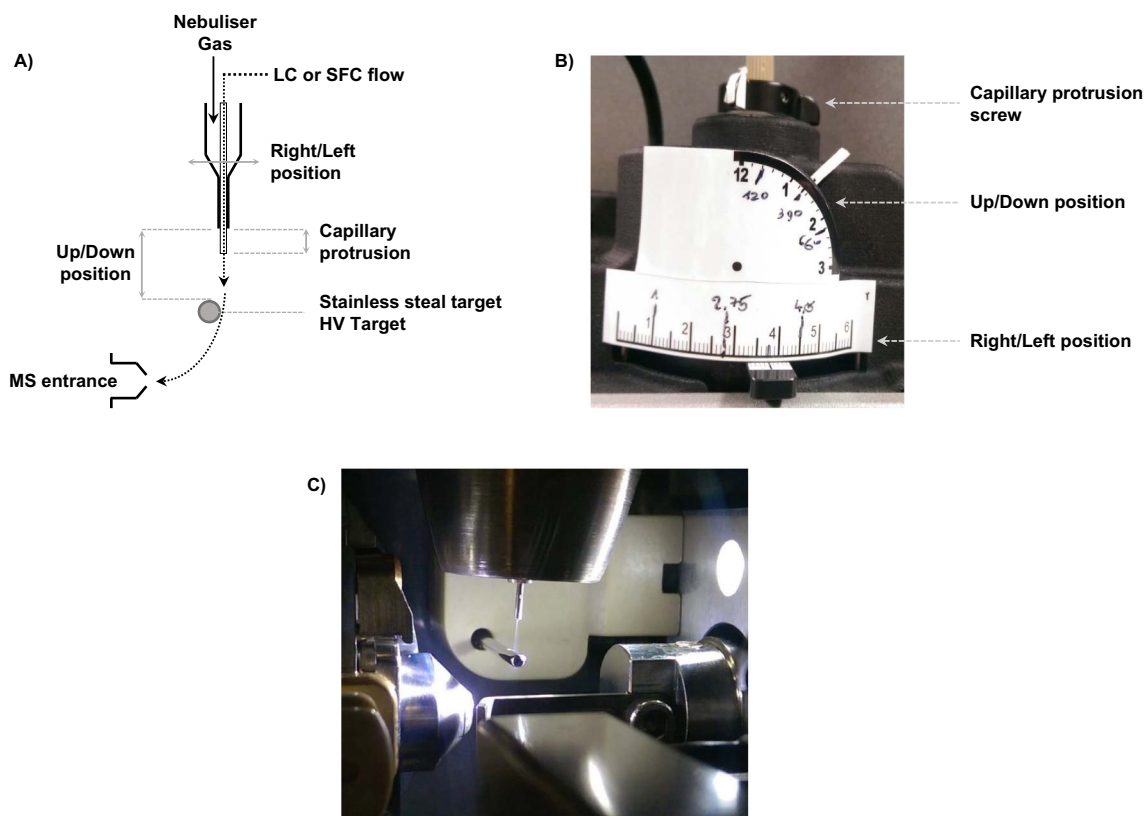


Fig. 1. UniSpray ionization source. A) Schematic representation of the UniSpray ionization source with position settings (HV, High Voltage; MS, Mass spectrometer); B) picture of the manual settings of the UniSpray ionization source; C) picture of the UniSpray ionization source.

mass spectrometry. Among them, electrospray ionization (ESI) is nowadays predominantly utilized interface, thanks to its wider range of applicability compared to atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) that are more dedicated to specific classes of analytes. Nevertheless, ESI still suffers from low ionization and low transmission efficiency [11].

Trying to improve ions transmission, a new API source design, known as UniSpray (Waters, Manchester, UK), was recently introduced. UniSpray source was developed to softly ionize analytes through an innovative API source design (Fig. 1) compatible with both UHPSFC and UHPLC effluents [11,12]. This new ionization source is close to ESI, but the ionization and aerodynamic mechanisms differ between these two interfaces [11]. In ESI, a high voltage is directly applied on the emitter capillary, thus generating a high velocity electrospray nebulizing the chromatographic effluent prior to the MS entrance. By contrast, in UniSpray ionization, the high voltage is applied onto a cylindrical stainless steel rod (target) directly located between the near-supersonic plume that emanates from the grounded capillary and the MS cone. More explanations on the UniSpray design can be found in [11,12]. In UniSpray, a better desolvation potentially increases the number of ions, droplets and charged droplets reaching the MS entrance compared to conventional API sources, leading to an enhanced sensitivity. Several physical parameters can be tuned on the UniSpray source, such as the protrusion of the capillary with respect to the concentric nebulizer capillary, the distance between the capillary and the high voltage target (HVT) as well as its right/left position of the capillary with respect to the HVT. All these physical parameters can be easily modified and affect the ionization efficiency of the source.

The goal of this study was to determine the optimal UniSpray settings using a design of experiment approach for maximizing sensitivity under UHPLC-MS and UHPSFC-MS conditions. Then, this ionization interface was tested for the analysis of a wide range of representative natural compounds belonging to different phytochemical classes. The

performance of the UniSpray ionization source was evaluated in terms of achievable sensitivity, applicability to a wide range of compounds having diverse physico-chemical properties, stability over time and matrix effects in plant extracts. The performance of UniSpray and ESI sources were systematically compared using the same HRMS device.

2. Material and methods

2.1. Chemicals and reagents

ULC/MS or CC/SFC grade methanol (MeOH), acetonitrile (MeCN), ethanol (EtOH), isopropanol (IPA), water (H₂O) and formic acid 99% (FA) were purchased from Biosolve (Valkenswaard, Netherlands). LC-MS grade ammonium formate (AmF) and leucine-enkephalin were purchased from Sigma-Aldrich (Buchs, Switzerland). A set of 120 natural compounds purchased from several providers, listed in supplementary data with their physicochemical properties (supplementary Table S1), was built according to their chemical diversity, to represent the natural compounds chemical space. The roots of *Glycyrrhiza glabra* L. were purchased from Dixa (St. Gallen, Switzerland).

2.2. Instrumentation

2.2.1. Liquid chromatography

Ultra-high performance liquid chromatographic (UHPLC) separations were performed on an Acquity I-class UPLC system (Waters, Milford, MA, USA) composed of a binary solvent delivery pump, an autosampler with flow through needle (SM-FTN) injection system and a column oven equipped with an active preheater. The autosampler temperature was fixed at 10 °C and the column was heated at 40 °C. Separations were performed on an Acquity UPLC HSS T3 C₁₈ column, 1.8 μm, 2.1 × 100 mm (Waters, Milford, MA, USA) in gradient mode using H₂O + 0.1% FA (solvent A) and MeCN + 0.1% FA (solvent B) as

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