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Application of a novel metabolomic approach based on atmospheric pressure photoionization mass spectrometry using flow injection analysis for the study of Alzheimer's disease

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ABSTRACT

The use of atmospheric pressure photoionization is not widespread in metabolomics, despite its considerable potential for the simultaneous analysis of compounds with diverse polarities. This work considers the development of a novel analytical approach based on flow injection analysis and atmospheric pressure photoionization mass spectrometry for rapid metabolic screening of serum samples. Several experimental parameters were optimized, such as type of dopant, flow injection solvent, and their flows, given that a careful selection of these variables is mandatory for a comprehensive analysis of metabolites. Toluene and methanol were the most suitable dopant and flow injection solvent, respectively. Moreover, analysis in negative mode required higher solvent and dopant flows (100 μl min^{-1} and 40 μl min^{-1}, respectively) compared to positive mode (50 μl min^{-1} and 20 μl min^{-1}). Then, the optimized approach was used to elucidate metabolic alterations associated with Alzheimer's disease. Thereby, results confirm the increase of diacylglycerols, ceramides, ceramide-1-phosphate and free fatty acids, indicating membrane destabilization processes, and reduction of fatty acid amides and several neurotransmitters related to impairments in neuronal transmission, among others. Therefore, it could be concluded that this metabolomic tool presents a great potential for analysis of biological samples, considering its high-throughput screening capability, fast analysis and comprehensive metabolite coverage.

1. Introduction

The main challenge in metabolomics is to obtain comprehensive and unbiased metabolic fingerprints of samples due to the huge heterogeneity and dynamism of metabolome [1]. In this context, mass spectrometry represents a very interesting analytical platform, since complexity of metabolome may be overcome through the use of complementary atmospheric pressure ionization methods [2]. Electrospray (ESI) is the most common ionization source employed in metabolomic studies because it is able to ionize compounds in a wide range of masses and polarities, and it may be coupled to liquid chromatography [3,4], capillary electrophoresis [5,6] or used by direct infusion mass spectrometry [7]. A second alternative is atmospheric pressure chemical ionization (APCI), more suitable for less polar compounds [8,9]. Finally, atmospheric pressure photoionization (APPI) complements ESI and APCI for the analysis of little polar or non-polar compounds, but it has been considerably less used in metabolomics. Atmospheric pressure photoionization is the most recent soft ionization technique for mass spectrometry, introduced simultaneously by Bruins and Syage in 2000 [10,11], extending the range of ionizable compounds to less polar ones, which are not readily ionized by ESI and APCI. Nevertheless, APPI is capable of ionizing both polar and non-polar compounds through proton transfer and charge exchange reactions respectively, so it could be considered a universal ionization source [12]. The APPI interface uses a photoionization lamp and a dopant flow to form dopant radical ions, which can directly ionize non-polar analytes through charge exchange reactions. On the other hand, for polar

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compounds dopant photoions can produce intermediate reactive species by reactions with solvent or oxygen molecules, in positive and negative ionization modes respectively, followed by a proton transfer reaction to the analyte [12]. Thereby, for the simultaneous analysis of both polar and non-polar compounds, the two reaction pathways must be accessible, which requires a careful selection of solvent, dopant, and their flows. Firstly, the carrier solvent employed can lead to a preferential mechanism, being favored charge exchange for low proton affinity solvents, whereas the addition of methanol or acetonitrile initiates proton transfer [13]. In the case of dopant, the most common reagent is toluene, but it may be not suitable for the ionization of non-polar compounds in high proton affinity solvents since it tends to transfer its proton to the solvent [14]. Alternatively, other dopants less reactive with the solvent have been proposed, such as anisole [15] or substituted benzenes [16]. Finally, the flow-rate of both solvent and dopant also play important roles in the photoionization process. In the case of solvent, ionization efficiency decreases when the flow increases, due to the formation of large unreactive protonated-solvent cluster ions, while signal increases with the dopant flow, until reach 5–10% of solvent flow [17]. Moreover, it has been reported that toluene can provide high ionization efficiency simultaneously to both polar and non-polar compounds delivered in reversed-phase solvents, simply limiting the solvent flow rate in order to avoid that reactions between toluene photoions and solvent were driven to completion [18].

In this way, APPI might present a considerable potential in metabolomics, not only because of its universality in terms of ionization capability, but also because is less susceptible to matrix effects and presents a linear dynamic range generally higher than that for ESI [19,20]. In addition, it requires less heat for desolvation than APCI allowing the analysis of thermally labile compounds [21]. APPI has been used for the analysis of many classes of compounds, including pharmaceutical drugs and metabolites [22], steroids [21], aldehydes and ketones [23] or pesticides [24]. However, only a few non targeted approaches include APPI as an alternative in metabolomics. Thereby, metabolomics based on liquid chromatography mass spectrometry and the integration of multiple ionization modes (ESI, APCI and APPI) has been previously proposed for the study of urine [25] and plasma samples [26], or to perform a more comprehensive analysis of lipidome [27–28]. Although APPI is normally coupled to liquid chromatography [29] or capillary electrophoresis [30], several reports confirm the ability of flow injection analysis for the determination of fullerenes and perfluorinated compounds [31], characterization of wine [32], olive oil [33] or Iberian ham [34], or the study of drugs [35] and petroleum [36]. This high-throughput approach exhibits several advantages in metabolomics such as fast and reproducible analysis, comprehensive metabolite coverage and simple data pre-processing [7], but it also presents important drawbacks associated with the lack of resolution for the differentiation of isobars and difficulty of quantification due to ion suppression. In order to overcome problems associated with isobaric interferences, the use of high resolution systems has become the main workhorse for accurate MS-fingerprinting, including time-of-flight (TOF), Fourier transform ion cyclotron resonance (FTICR), and especially the hybrid system Q-TOF, which allows more accurate mass measurement than single TOF instruments and structural elucidation by MS/MS experiments [37]. On the other hand, although ion suppression is a potential problem in any MS-based metabolomic platform, there is no evidence that it presents a more detrimental effect on flow infusion fingerprinting than in hyphenated approaches [7]. In fact, the large amount of different compounds in biological samples helps to regulate matrix effects, so that ion suppression becomes a constant factor imposed uniformly in all samples derived from similar types of tissues [38]. Thereby, MS-fingerprinting has proved to be an excellent tool for high-throughput metabolomic characterization of complex samples, usually using electrospray ionization, as recently reviewed by Draper et al. [7]. However, to date, the use of FIA-APPI-MS has not been considered in metabolomic analysis.

This work explores for the first time the potential of flow injection analysis and high resolution tandem mass spectrometry with atmospheric pressure photoionization source (FIA-APPI-QTOFMS) in metabolomics. Several critical parameters were optimized for the simultaneous analysis of both polar and non-polar compounds in positive and negative ionization modes, such as type of dopant, flow injection solvent and their working flows. For this purpose, a test mixture of representative metabolites from human serum was used. Then, the optimized approach was applied to blood serum samples in order to investigate metabolic abnormalities associated with Alzheimer's disease (AD). This neurodegenerative disorder is poorly understood, and its etiology is still unknown, although it is likely to be a conglomeration of different pathological entities. There is currently no cure for Alzheimer's disease, but early diagnosis could help monitor disease progression and target therapies earlier in the course of the disease, so identification of reliable biomarkers is becoming increasingly important. Multivariate statistics demonstrated the ability of this high-throughput metabolomic tool for discriminating between AD patients and healthy controls, and allowed the identification of different metabolic failures underlying to pathological features of this health disorder.

2. Material And methods

2.1. Chemicals and samples

The solvents (HPLC-grade) methanol, ethanol, chloroform, dichloromethane, acetonitrile and isopropanol were purchased from Fisher Scientific (Leicestershire, UK), while dopants toluene, anisole and chlorobenzene were supplied by Aldrich (Steinheim, Germany). Water was purified with a Milli-Q Gradient system (Millipore, Watford, UK). Standards of L-glutamine, L-valine, L-cysteine, L-aspartic acid, L-arginine, L-histidine, L-glutamic acid, L-phenylalanine, D-glucose, creatine, creatinine, cholesterol, di-oleoyl-phosphocholine and triolein were from Sigma Aldrich. Blood samples were collected in the morning by puncture of the antecubital vein and collected in BD Vacutainer SST II tubes with gel separator and Advance vacuum system. The samples were immediately cooled and protected from light for 30 min, and after centrifugation (3500 rpm for 10 min) serum was aliquoted and frozen at -80 °C until analysis. Alzheimer's disease patients (AD, $N=30$, age 80.3 ± 5.0, male/female 12/18) were newly diagnosed of sporadic Alzheimer's disease by the Neurologic Service of Hospital Juan Ramón Jiménez (Huelva, Spain), according to the criteria of the NINCDS-ADRDA [39], and only subjects that had not yet received any type of medication were included in the study. Healthy controls (HC, $N=30$, age 73.5 ± 5.9, male/female 10/20), who had not more than two reported cases of Alzheimer's disease in their families, were studied by neurologists to confirm the absence of neurological disorders. Demographic characteristics of groups considered in the study are listed in the Supporting information, including age, gender, co-morbidities, medication and family history of AD. It is noteworthy that most subjects suffered other co-morbidities and were under different medical treatments, but there were not significant differences among the groups considered. The study was performed in accordance with the principles contained in the Declaration of Helsinki and approved by the Ethical Committee of University of Huelva.