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Anionic metabolite profiling by capillary electrophoresis–mass spectrometry using a noncovalent polymeric coating. Orange juice and wine as case studies

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ABSTRACT

In several metabolomic studies, it has already been demonstrated that capillary electrophoresis hyphenated to mass spectrometry (CE–MS) can detect an important group of highly polar and ionized metabolites that are overseen by techniques such as NMR, LC–MS and GC–MS, providing complementary information. In this work, we present a strategy for anionic metabolite profiling by CE–MS using a cationic capillary coating. The polymer, abbreviated as PTH, is composed of a poly-(N,N,N',N'-tetraethyldiethylenetriamine, N-(2-hydroxypropyl) methacrylamide, TEDETAMA-co-HPMA (50:50) copolymer. A CE–MS method based on PTH-coating was optimized for the analysis of a group of 16 standard anionic metabolites. Separation was achieved within 12 min, with high separation efficiency (up to 92,000 theoretical plates per meter), and good repeatability, namely, relative standard deviation values for migration times and peak areas were below 0.2 and 2.1%, respectively. The optimized method allowed the detection of 87 metabolites in orange juice and 142 metabolites in red wine, demonstrating the good possibilities of this strategy for metabolomic applications.

1. Introduction

Among the different mass spectrometry (MS)-based approaches used in metabolomics, those that combine a physical/chemical separation/fractionation prior to MS analysis are the most widely used. Thus, although at the expenses of less high-throughput performance, hyphenation of high-resolution separation techniques and MS is usually carried out in order to avoid sample matrix effects while revealing the metabolome of a biological system as much as possible. The majority of applications in the field of metabolomics over the last years have used liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS). However, ionized, highly polar and hydrophilic compounds are poorly retained in reversed-phase (RP) LC–MS. Hydrophilic interaction chromatography–MS (HILIC–MS) allows profiling of polar compounds, providing complementary metabolic information to RPLC, as has been previously demonstrated [1]. It should be mentioned that re-equilibration of HILIC columns is slow particularly with gradient elution of mobile phases containing buffers while resolution in HILIC is not as high as in RPLC. Capillary electrophoresis–mass spectrometry (CE–MS) is considered a powerful analytical tool for the analysis of charged and highly polar metabolite species [2,3] that provides complementary information to RPLC–MS and HILIC–MS [4–6]. Up to date, the number of applications involving the analysis of cationic metabolites and CE–MS in positive ionization mode far exceeds the number of works involving anionic metabolite profiling [7]. This is mainly due to the fact that in CE–MS the outlet of the capillary is not immersed in the vial electrolyte, thus, by using a fused silica capillary and normal polarity, the electroosmotic flow (EOF) moves from the anode to the cathode (where the MS detector is located), and the CE electrical current is stabilized. Additionally, rapid analysis with good resolution is obtained since both EOF and the electrophoretic mobility of cationic metabolites are toward the cathode (MS detector). In contrast, by using a fused silica capillary and reversed polarity for anionic compounds analysis, the direction of the EOF is opposing to the MS detector, and thus, unstable electrical connection between the tip of the capillary and the grounded electrospray needle, is

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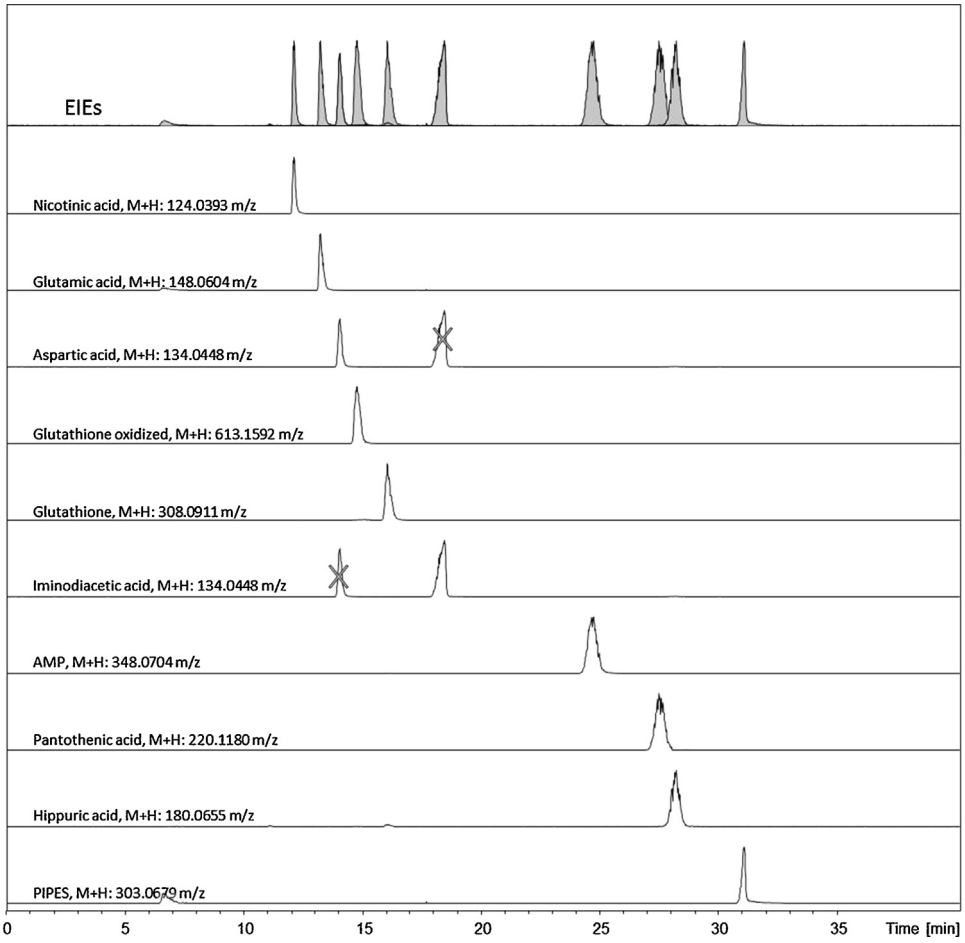

Fig. 1. CE–ESI–TOF MS extracted ion electropherograms (EIEs) of metabolites in mix of anionic metabolites (ARM) separated using 1 M formic acid as BGE at normal CE polarity (+20 kV) in a bare silica capillary and positive ion ESI–MS mode. Sheath liquid: 2-propanol–water (50:50, v/v) at 0.24 mL/h. Other analytical conditions are described in Section 2.4.

typically observed [8]. However, obtaining metabolome information as complete as possible requires the combination of several methodologies, inherent to the highly diverse chemical structure of metabolites. For anionic metabolites, CE under alkaline background electrolyte (BGE) conditions has been demonstrated to be a useful approach when no MS detection is carried out. When alkaline BGEs are used, low signals were observed for anionic test mixtures [9]. Profiling of anionic compounds for metabolomic applications has been achieved by pressure assisted CE using fused-silica [10], inert polymer capillaries [8] or non-charged polymer inner surface coating [11]. On the other hand, since the very first work by Tsuda [12] who described the use of the cationic surfactant cetyltrimethylammonium bromide (CTAB) added to the separation buffer to reverse the EOF, a variety of cationic surfactant additives have been developed. However, this procedure should generally be avoided when working with MS detection since these surfactants suppress sample ionization and contaminate the ion source. An interesting strategy in CE to reverse the EOF toward the anode (the MS direction when working in reversed polarity), to prevent current drops, is the use of physically adsorbed coating materials. Following this strategy, just by rinsing the capillary with a solution containing the coating agent, the coated capillary can be easily prepared. In this work a polymer bearing a dendronic triamine derived from N,N,N',N'-tetraethyldiethylenetriamine (TEDETA) moiety, which is linked to a unit through a spacer, (unit labeled as TEDETAMA), was copolymerized together with neutral hydrosoluble units of hydroxypropylmethacrylamide (HPMA) at a 50:50 molar percentage. This copolymer is tested for the first time as a robust coating to reverse EOF for anionic metabolite profiling by CE–MS. The feasibility of the polymer-coated capillary approach was corroborated via the analysis of anionic metabolites of complex samples such as orange juice and red wine.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent grade and used as received. Standard metabolites were from Sigma–Aldrich (St. Louis, MO, USA). All reagents and solvents employed in the preparation of CE electrolytes and sheath liquids were of MS grade: methanol and 2-propanol were from Sigma–Aldrich (St. Louis, MO, USA). Ammonium hydroxide, formic acid and acetic acid were from Fluka (Buchs, Switzerland). Ammonium acetate, ammonium formate and boric acid were purchased from Merck (Darmstad, Germany). The distilled water was deionized using a Milli-Q system from Millipore (Bedford, MA, USA). An aqueous solution containing 0.1 M of sodium hydroxide, from Panreac Quimica S.A. (Barcelona, Spain) was used to rinse the capillary.

2.2. Metabolite test mixtures and sample preparation

Cationic metabolite-rich mixture (CRM) was composed of 0.5 mM of: L-carnosine, L-anserine, L-ornithine,