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Short communication

Capillary electrophoresis coupled to mass spectrometry employing hexafluoro-2-propanol for the determination of nucleosides and nucleotide mono-, di- and tri-phosphates in baby foods

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ABSTRACT

The present work describes a method for the simultaneous determination of unmodified nucleosides and nucleotide mono-, di- and tri-phosphates by capillary electrophoresis coupled to mass spectrometry (CE-MS). The use of hexafluoro-2-propanol (HFIP) in the separation medium, and as an additive to the sheath liquid of the electrospray interface (ESI), generated a highly efficient and sensitive method. Instrumental limits of detection in the range of 14–53 ng mL^{-1} for nucleosides and 7–23, 20–49 and 64–124 ng mL^{-1} for nucleotide mono-, di-, and tri-phosphates, respectively, were found. Sample treatment involved diluting an aliquot of baby food with ultra-high quality water and applying centrifugation-assisted ultrafiltration (CUF). The proposed method was validated and used to analyse a variety of baby food samples (16 in total) such as fish, meat, fruits, and baby dairy desserts that may endogenously contain these analytes.

1. Introduction

Nucleosides (NUs) and nucleotides are essential building blocks of DNA and RNA and also play an important role in energy metabolism. The determination of these molecules is of special interest (Studzinska & Buszewski, 2013) in biochemistry (Bolin & Cardozo-Pelaez, 2007), medicine (Yeung, Ding, & Casley, 2008), food microorganisms (Zhu, Wang, Wang, Zhou, & Shi, 2016) and food analysis (Chen, Yang, Wang, & Li, 2010; Viñas et al., 2009; Wu et al., 2015; Yamaoka et al., 2010).

Very few liquid chromatographic (LC) applications have been described for the simultaneous separation and detection of nucleosides and nucleotides, which are also often limited owing to the excessively long time required for separation (Ferreira, Mendes, Gomes, Faria, & Ferreira, 2001; Gill & Indyk, 2007; Ranogajec, Beluhan, & Šmit, 2010; Wu et al., 2015; Yamaoka et al., 2010). The adsorption of the phosphorylated nucleotides on the surface of the particles supporting reversed stationary phases, and on the metallic surfaces of the LC–MS setup, makes chromatographic separations and MS detection difficult (Kim, Campo, & Smith, 2004; Tuytten et al., 2006). Recently, our group developed a method for the determination of nucleosides and nucleotides based on hydrophilic interaction chromatography with ion-pairing reagents (IP-HILIC-MS/MS) (Mateos-Vivas, Rodríguez-Gonzalo, García-Gómez, & Carabias-Martínez, 2015) with a triple quadrupole MS detector. In this method, the main drawback was that the injection medium required a high percentage of acetonitrile to achieve the appropriate retention in the HILIC stationary phase. Therefore, in its application to the analysis of food with a high water content, the dilution of the food extracts with acetonitrile prior to being injected into the HILIC-MS/MS system was required.

Regarding CE, methods have been described for the determination of nucleosides and nucleotides in food materials (Chen et al., 2010; Rodríguez-Gonzalo, Domínguez-Álvarez, Mateos-Vivas, García-Gómez, & Carabias-Martínez, 2014; Mateos-Vivas, Rodríguez-Gonzalo, Domínguez-Álvarez, et al., 2015). Soga et al. (2007) have proposed a CE-MS method for the determination of three nucleotide mono-, di- and tri-phosphates (NMPs, NDPs, NTPs), among other analytes employing a common fused silica CE capillary. They observed adsorption phenomena between the phosphorylated nucleotides and the silanol groups on the inner wall surface of the capillary. In an attempt to prevent this, a dynamic coating with a phosphate electrolyte was successfully applied prior to the injection of the samples containing the analytes. Liu, Huang, Tyrrell, and Dovichi (2005) reported the use of CE-ESI-MS for the determination of antiretroviral dideoxynucleosides (ddNs) and a set of ribonucleosides and ribonucleotides in a

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human hepatocellular cell line based on a basic ammonium acetate buffer suitable for ESI-MS detection.

Hexafluoro-2-propanol (HFIP) has been used as a modifier of vesicle systems (Tian, Li, Mei, Deng, & Xiao, 2015) and together with Gemini pyrrolidinium surfactants in phosphate buffer has been employed as a dynamic coating in the CE-DAD separation of proteins (Tian, Li, Mei, Cai et al., 2015). In addition, due to its behaviour as a strong H-bond, HFIP seems to be an excellent coacervation-inducing agent and has been used to produce a phase separation in micellar systems, taking advantage of its capacity to separate and preconcentrate strongly polar analytes (Chen, Zhang, Li, Mei, & Xiao, 2014). As far as we know HFIP has not been utilized as a component of the background electrolyte (BGE) in capillary zone electrophoresis or as an additive in the sheath liquid used in CE-MS.

One of the objectives of this work was to develop an alternative method, based on CE-MS, for the simultaneous determination of unmodified NUs and NMPs, NDPs and NTPs capable of avoiding interactions between phosphorylated compounds and different parts of the instrumental CE-MS device. In the present work, preliminary studies showing the advantages associated with the use of HFIP in the BGE and in the sheath liquid of the ESI are described for the first time. The main goal of this work was to develop a simple, efficient and validated analytical method to determine endogenous NUs and nucleotides in baby foods with fish, meat or fruit and in baby dairy desserts.

2. Experimental

2.1. Chemicals and reagents

Target compounds (Padivitage, Dissanayake, & Armstrong, 2013) were guanosine (G), uridine (U), inosine (I); and mono- di- and tri-phosphate of adenosine (AMP, ADP, ATP), cytidine (CMP, CDP, CTP), guanosine (GMP, GDP, GTP), inosine (IMP, IDP, ITP) and uridine (UMP, UDP, UTP), respectively, and have been described in a previous paper (Mateos-Vivas, Rodríguez-Gonzalo, García-Gómez et al., 2015). Stock solutions containing the nucleosides and nucleotides reference standards, at 500 μg mL^{-1}, were prepared in ultra-high quality (UHQ) water. All solutions were kept at 4 °C in amber glass bottles, and were stable for at least 6 months. Working solutions were prepared daily by mixing the appropriate amounts of the stock solutions and diluting in UHQ water.

Hexafluoro-2-propanol was provided by Sigma-Aldrich, the reagent grade 25% (w:w) concentrated ammonia and HPLC grade isopropanol were purchased from Scharlau, and the UHQ water was obtained using a Wasserlab (Noain, Spain) ultramatic water purification system.

2.2. Instrumentation

The CE-MS instrumentation, fused-silica capillaries (50 μm I.D.), centrifugal ultrafiltration (CUF) devices and the non-thermostated centrifuge used have been described in a previous paper (Rodríguez-Gonzalo et al., 2014).

2.3. CE separation conditions

Standard solutions were injected using a pressure of 50 mbar over 40 s. The injection medium for the reference standards was UHQ water, and the other samples were baby food extracts obtained from the CUF device; all samples contained 80 μL mL^{-1} of concentrated ammonia solution. Electrophoretic separation was achieved with a voltage of 30 kV (positive CE mode), with an initial ramp of 7 s. The BGE was prepared as follows: an appropriate volume of liquid HFIP reagent was added to UHQ water, the pH was adjusted to 10.2 by addition of drops of concentrated ammonia solution reagent grade and diluted in a 10.0 mL volumetric flask to the mark with UHQ water. The temperature of the capillary was kept constant at 35 °C. Before the first use, the capillary was conditioned by flushing with the BGE for 5 min at 8 bar. This procedure was also implemented as a daily start-up routine. After each run of the standards, the capillary was prewashed at 5 bar for 3 min with fresh BGE, and after each run of baby food samples the capillary was prewashed at 8 bar for 1 min with aqueous HFIP solution 20% (v:v) followed by 8 bar 1 min and 5 bar 2 min of fresh BGE.

2.4. ESI-MS conditions

MS was performed using an Agilent MSD SL mass spectrometer equipped with a single quadrupole analyser. An Agilent coaxial sheath-liquid sprayer was used for the ESI interface in CE-MS coupling. The sheath liquid was 1.6% (v:v) of HFIP in UHQ water and was delivered at a flow rate of 1.00 mL min^{-1} by an Agilent 1100 series pump equipped with a 1:100 flow-splitter. The ESI(−) voltage was set at 3500V. The other optimized electrospray parameters were: drying-gas flow rate, 7.0 L min^{-1}; drying-gas temperature, 350 °C and a programmed nebulizing-gas pressure (PNP) of 7 psi from the moment of injection until after the electroosmotic flow had reached the end of the CE capillary (about 5.7–6.0 min). Then, the nebulizing-gas pressure was set to its optimal level of 12 psi to get the best signals for the analytes. The mass spectrometer was operated in the negative-ion mode (ESI−). The optimized fragmentor voltage was set to 190V for all analytes, except for the three NUs where the voltage was set to 110 V. For quantification, the selected ion monitoring (SIM) mode was utilized, the *m/z* corresponding to the $[M-H]^-$ ions with a peak width of 0.05 min. A detection window was achieved where NUs were monitored with a dwell time of 97% and the combined nucleotides were monitored with a dwell time of 17%.

2.5. Samples and sample treatment

Sixteen baby food samples of different brands and ingredients were analysed. Four of the samples contained fish (9–16% of the total composition) and six samples contained meat (10–15% of the total composition). A third group was comprised of four baby foods made up of almost 100% fruit, and a fourth group comprised two baby food desserts containing yogurt for babies and a cereal yogurt.

The proposed sample treatment method was performed as follows: 0.50 g of the sample was weighed in a 50-mL centrifuge tube and UHQ water was added to obtain a final weight of 4.5 g. The sample was shaken manually and then centrifuged at room temperature for 15 min (2800*g*). A 4.0-mL aliquot of the supernatant was collected, avoiding the solid residue at the bottom of the tube, and passed through a CUF device (30 min, 2800*g*) previously conditioned with 5.0 mL of UHQ water (15 min, 2800*g*). Afterwards, without removing either the filtrate or the any remaining liquid, 0.5 mL of UHQ water was added to the CUF device and centrifuged again (30 min, 2800*g*), carrying out an extra step of washing the filter. The filtrate was directly analysed using CE-MS as described in Sections 2.3 and 2.4.

2.6. Method validation and sample quantification

The quantitative variable was the peak area generated by the respective deprotonated ion $[M-H]^-$ expressed in arbitrary peak area units (SIM acquisition mode). The method was validated using the sole with béchamel sample, evaluating the following parame-