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Ionic liquids as mobile phase additives in high-performance liquid chromatography with electrochemical detection: Application to the determination of heterocyclic aromatic amines in meat-based infant foods

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ABSTRACT

The beneficial effects of several ionic liquids (ILs) as mobile phase additives in high-performance liquid chromatography with electrochemical detection for the determination of six heterocyclic aromatic amines (HAs) have been evaluated for first-time. The studied ionic liquids were 1-butyl-3-methylimidazolium tetrafluoroborate (BMIm-BF_4), 1-hexyl-3-methylimidazolium tetrafluoroborate (HMIm-BF_4) and 1-methyl-3-octylimidazolium tetrafluoroborate (MOIm-BF_4). Several chromatographic parameters have been evaluated in the presence or absence of ILs, or using ammonium acetate as the most common mobile phase additive, with three different C18 stationary phases. The effect of the acetonitrile content was also addressed. In general, best resolution, lower peak-widths (up to 72.1% lower) and lower retention factors are obtained when using ILs rather than ammonium acetate as mobile phase additives. The main improvement was obtained in the baseline noise, being 360% less noisy for BMIm-BF_4, 310% for HMIm-BF_4, and 227% for MOIm-BF_4, when compared to ammonium acetate at +1000 mV. Different chromatographic methods using the best conditions for each IL were also evaluated and compared. Finally, the best chromatographic conditions using 1 mM of BMIm-BF_4 as mobile phase additive, the Nova-Pak® C18 column, 19% (v/v) of acetonitrile content in the mobile phase, and +1000 mV in the ECD, have been applied for the chromatographic analysis of six HAs contained in meat-based infant foods. The whole extraction method of meat-based infant foods using focused microwave-assisted extraction and solid-phase extraction has also been optimized. Extraction efficiencies up to 89% and detection limits ranged between 9.30 and 0.165 ng g^{-1} have been obtained under optimized conditions.

1. Introduction

The separation of basic compounds in high-performance liquid chromatography (HPLC) still remains problematic due to the silanol interactions [1,2]. The poor performance seen with basic compounds has been partially addressed through the development and introduction of the so-called base-deactivated materials [3,4]. Nevertheless, addition of alkylamines and other amino quenchers to the mobile phase does not fully remove the deleterious effect of free silanols on the retention of basic analytes, even when employing the purified and least acidic silica supports [5].

A new alternative to reduce or suppress the silanol activity is based on the utilization of ionic liquids (ILs) as additives of the mobile phase [6–13]. ILs are a class of low melting point ionic compounds which have a variety of properties allowing many of them to be sustainable green solvents. ILs possess high thermal stabilities and negligible vapor pressures making them attractive alternatives to environmentally unfriendly solvents that produce volatile organic compounds [14]. They have been used in a number of analytical applications, including some extraction processes [15–19].

ILs evidently lose many of their original properties when they are diluted to act as eluent additives. In some cases, they may keep several of their intermolecular interactions, which may be useful for chromatographic separations [9]. The beneficial effects of ILs in the separation of basic compounds have been described as it follows: ILs cations could interact and compete for the silanol groups with the basic groups of the analytes. In addition, the nonpolar alkyl groups of the stationary phase can interact with different alkyl groups of the heterocyclic ring or quaternary cation of the IL [6]. At the same time, the chaotropic character of the anions constituting ionic liquids is responsible for possible ion-paring with cationic solutes [10]. All these interactions could efficiently shield the residual silanols and improve the peak shapes while reducing the chromatographic retention times of the basic analytes [9].

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All the reported applications of ILs as mobile phase additives include HPLC with UV or diode array detection [6–8,10]. To our knowledge, there are not reported studies using HPLC with electrochemical detection (ECD) and ILs. Electrochemical detection of analytes is based on the electrochemical response of the compounds at the operating potential, and it requires the presence of an ionic mobile phase in order to have an adequate electrolyte solution. Therefore, it results necessary to evaluate the effect of the ionic liquids in such ionic mobile phase.

The main aim of this work was to study for first-time the beneficial effect of a group of alkylimidazolium-based ILs as mobile phase additives in HPLC with ECD in the determination of a group of heterocyclic aromatic amines (HAs). HAs are known for their potent mutagenic response and they have also been linked to cancer [20–22]. These basic compounds are usually determined by HPLC using triethylamine (TEA) [23] or ammonium acetate [24,25] as mobile phase additives. Several specific columns have been developed to analyze these amines [26], and in this way, this study includes the effect of ILs in three different C18 stationary phases (with low and moderate silanol activity). Several chromatographic parameters including resolution, efficiency, peak-width, peak-area, peak-height, and retention factor have been evaluated in the presence or absence of ILs. Furthermore, both the influence of the acetonitrile content and the effect of the ILs on the relationship between the applied potential *versus* the obtained intensity of the ECD, have been treated. Finally, an application of the method using the best chromatographic conditions and the most adequate IL (1-butyl-3-methylimidazolium tetrafluoroborate, BMIm-BF_4) has been conducted in meat-based infant foods to determine six HAs. The overall extraction process using focused microwave-assisted extraction and solid-phase extraction in combination with HPLC–ECD has also been optimized. ILs are also proposed for first time as eluting agents in SPE.

2. Experimental

2.1. Reagents

The studied amines were: 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), and 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), purchased from Toronto Research Chemicals (North York, ON, Canada); 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 9*H*-pyrido[4,3-*b*]indole (NH), and 1-methyl-9*H*-pyrido[4,3-*b*]indole (H), purchased from Aldrich-Chemie (Beerse, Belgium). Stock standard solutions in methanol were prepared containing 92 μg mL^{-1} of H; 520 μg mL^{-1} of NH; 400 μg mL^{-1} of Trp-P-2, Trp-P-1, AαC and MeAαC. These solutions were kept refrigerated at 0 °C and protected from light, and they were used for the preparation of working standard solutions.

The studied ionic liquids were: 1-butyl-3-methylimidazolium tetrafluoroborate (BMIm-BF_4), 1-methyl-3-octylimidazolium tetrafluoroborate (MOIm-BF_4), and 1-hexyl-3-methylimidazolium tetrafluoroborate (HMIm-BF_4), purchased from Fluka (Buchs, Switzerland).

Ethylenediaminetetraacetic acid (EDTA), ammonium acetate and potassium chloride, were of pro-analysis grade quality and supplied by Merck (Darmstadt, Germany). Acetonitrile and methanol were of HPLC-grade supplied by Scharlau (Barcelona, Spain) and Merck, respectively. Water was purified using a Milli-Q gradient A10 system from Millipore (Billerica, MA, USA). All solvents were filtered through a 0.45 μm Durapore® membrane filter (Millipore) before being used in the chromatographic system.

2.2. Instrumentation

Focused microwave-assisted extractions were performed at atmospheric pressure using the Discover model of the CEM Focused Microwave™ Synthesis System (Matthews, NC, USA) equipped with an infrared temperature control system, stirring and cooling options. The ChemDriver™ software (CEM) was used for data acquisition.

C18 cartridges (100 mg) were purchased from Supelco (Bellefonte, PA, USA). The cartridges were conditioned using 5 mL of methanol, followed by 5 mL of water and then 500 μL of ammonium acetate. Elution was accelerated with a Visiprep™ solid-phase extraction vacuum manifold from Supelco.

The HPLC system was a liquid chromatograph consisting of a solvent delivery system ProStar 230 from Varian (Palo Alto, CA, USA) equipped with a Rheodyne valve (Supelco) with a 20 μL injection loop. The detection of HAs was carried out using a ProStar 370 Electrochemical Detector (Varian). It was provided with a working electrode (glassy carbon), a reference electrode (Ag/AgCl, 2 M) and an auxiliary electrode (stainless steel). Three analytical columns were used: a TSK Gel® ODS-80TM column (5 mm, 150 mm × 3.9 mm i.d.) from Tosoh Biosep (Stuttgart, Germany), a Nova-Pak® C18 column (4 mm, 150 mm × 3.9 mm i.d.) from Waters (Milford, MA USA), and a ABZ+ plus column (5 mm, 150 mm × 2.1 mm i.d) from Supelco. All these columns were used in combination with a Pelliguard LC-18 guard column (Supelco). Data were acquired with the Star 5.51 chromatography workstation software (Varian).

2.3. Focused microwave-assisted extraction and solid-phase purification procedures

Meat-based infant foods samples were bought in a local supermarket, and used without being subjected to any further manipulation. The spiking process of infant foods was as it follows: the spiking solution was slowly added to form a dough which was mechanically stirred for several minutes. Afterwards, it was stored in the dark for 24 h. The spiked levels were: 1.45 μg g^{-1} for NH, 0.490 μg g^{-1} for H, 0.443 μg g^{-1} for Trp-P-2, 1.40 μg g^{-1} for Trp-P-1, 1.38 μg g^{-1} for AαC, and 0.583 μg g^{-1} for MeAαC.

The optimum extraction process of the samples was as it follows: a 6 mL aliquot of extractant phase (composed by 20%, v/v of methanol in 0.8 M of NaOH) was added to 1 g of infant food sample (spiked or non-spiked), and placed in a Pyrex® tube of 40 mL. After ensuring that an agitation bar was placed in the tube, the extraction tube was introduced into the microwave cavity. The microwave-assisted extraction step was conducted as described in a previous work [25]. Afterwards, the tube was allowed to cool at room temperature and was placed in a water-ice bath for several minutes. This purification step was required for the extraction of lipids and fats, and it has been described in several works [25,27]. Then, the supernatant was introduced in a tube, and centrifuged during 20 min at 4000 rpm. 4 mL of the centrifuged supernatant were then diluted at 20 mL with ammonium acetate 0.5 M, and subjected to the SPE purification step.

The optimized procedure with the C18 SPE cartridge was as it follows: the diluted phase with ammonium acetate coming from the previous step (20 mL) was then loaded on the C18 (100 mg SPE) cartridge. This SPE cartridge was then washed with 5 mL of Milli-Q water, and eluted with 1 mL of the mixture 10 mM BMIm-BF_4:acetonitrile (1:1). An aliquot of 20 μL of this eluted extract was then directly injected in the HPLC system.

2.4. HPLC–ECD procedure

The working potential of the electrochemical detector was set at +1000 mV. The chromatographic separation of the HAs was car-