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Optimizing separation conditions of 19 polycyclic aromatic hydrocarbons by cyclodextrin-modified capillary electrophoresis and applications to edible oils

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ABSTRACT

For the first time, the separation of 19 polycyclic aromatic hydrocarbons (PAHs) listed as priority pollutants in environmental and food samples by the United States Environmental Protection Agency (US-EPA) and the European Food Safety Authority was developed in cyclodextrin (CD)-modified capillary zone electrophoresis with laser-induced fluorescence detection (excitation wavelength: 325 nm). The use of a dual CD system, involving a mixture of one neutral CD and one anionic CD, enabled to reach unique selectivity. As solutes were separated based on their differential partitioning between the two CDs, the CD relative concentrations were investigated to optimize selectivity. Separation of 19 PAHs with enhanced resolutions as compared with previous studies on the 16 US-EPA PAHs and efficiencies superior to 1.5×10^5 were achieved in 15 min using 10 mM sulfobutyl ether-β-CD and 20 mM methyl-β-CD. The use of an internal standard (umbelliferone) with appropriate electrolyte and sample compositions, rinse sequences and sample vial material resulted in a significant improvement in method repeatability. Typical RSD variations for 6 successive experiments were between 0.8% and 1.7% for peak migration times and between 1.2% and 4.9% for normalized corrected peak areas. LOQs in the low μg/L range were obtained. For the first time in capillary electrophoresis, applications to real vegetable oil extracts were successfully carried out using the separation method developed here.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) form a large group of about 10,000 compounds with two or more fused aromatic rings. Their origin is both anthropogenic (incomplete combustion of organic matter such as oil, wood or fossil fuels due to human activity) [1,2] and natural (e.g. forest fires and volcanoes). However, PAHs are known to have carcinogenic and mutagenic effects caused by the binding of their metabolites to DNA [3]. The exposure of humans to these compounds creates health risks, especially with food contaminated by PAHs coming from environment or production practices (smoking, heating, and drying) [4–7].

Some decades ago, the United States Environmental Protection Agency (US-EPA) established a list of priority pollutants: 'the 16 US-EPA PAHs' [8]. Moreover in 2002, the Scientific Committee on Food of the European Commission (today replaced by the European Food Safety Authority (EFSA)) published another list of 15 priority PAHs for monitoring the contamination of food products, including some compounds of the US-EPA list [9]. Later, in 2008, a 16th compound (benzo(c)fluorene, BcFLR) was officially included into the EU priority PAHs by the EFSA [10]. This new list is commonly called '15+1 EU priority PAHs', so that it can be distinguished from the 16 US-EPA

Abbreviations: ACP, acenaphthene; ACY, acenaphthylene; ANT, anthracene; BaA, benzo(a)anthracene; BbFA, benzo(b)fluoranthene; BjFA, benzo(j)fluoranthene; BkFA, benzo(k)fluoranthene; BcFLR, benzo(c)fluorene; BghiP, benzo(ghi)perylene; BaP, benzo(a)pyrene; CZE, capillary zone electrophoresis; CHR, chrysene; CD, cyclodextrin; CPcdP, cyclopenta(c-d)pyrene; DS, degree of substitution; DBahA, dibenzo(a,h)anthracene; DBaeP, dibenzo(a,e)pyrene; DBahP, dibenzo(a,h)pyrene; DBaiP, dibenzo(a,i)pyrene; DBalP, dibenzo(a,l)pyrene; EFSA, European Food Safety Authority; FA, fluoranthene; FLR, fluorene; IP, indeno(1,2,3-cd)pyrene; LIF, laser-induced fluorescence; MeOH, methanol; MCH, 5-methylchrysene; Me-β-CD, methyl-β-CD; NPH, naphthalene; PHE, phenanthrene; PAH, polycyclic aromatic hydrocarbons; Pyr, pyrene; SBE-β-CD, sulfobutyl ether-β-cyclodextrin; US-EPA, United States Environmental Protection Agency

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PAHs. Eight PAHs known to be mutagenic or carcinogenic are in common between the two priority lists, resulting in 24 PAHs under regulations (see Supplementary Data).

To determine complex mixtures of PAHs at low concentrations in food and environmental samples, reliable analytical methods are needed [11,12]. Analytical procedures to quantify PAHs are mostly based on liquid chromatography coupled to diode array detector [13–15] or fluorescence detector [15–18] and gas chromatography coupled to mass spectrometry [19–22]. Capillary electrophoresis (CE) with its high separation efficiencies, low reagent and sample consumption, speed of analysis, and easier transfer to chip format, is an interesting alternative to previous chromatographic methods [23–25]. Capillary zone electrophoresis (CZE), the most classical form of CE, however, is not suited for the analysis of such neutral and hydrophobic compounds, but micellar electrokinetic chromatography (MEKC) is well adapted [26–28]. In this case, micelles formed from surfactants allow both the separation of PAHs and the increase in their solubility in the aqueous electrolyte. However, the addition of a micellar phase alone usually does not provide enough selectivity to separate a large number of PAHs, since they are too strongly associated with the micelles. Usually, the addition of modifiers, such as organic solvents or cyclodextrins (CDs), to the buffer is necessary [29–35].

In MEKC, the addition of CDs is the most successful strategy to improve method selectivity. Thanks to their ability to form host–guest inclusion complexes with hydrophobic compounds, the partitioning of components between the micellar and aqueous/CD phases is modified [36,37]. However, CD-modified MEKC suffers from a lack of selectivity evidenced by long analysis times and co-migrations of similar PAHs [38].

Electrochromatography (CEC) can also be employed for PAH separations but it appears in the literature that PAHs are often used as model compounds to characterize the CEC performance of the stationary phases: packed- or monolithic-based ones [39–41]. Applications to complex PAH mixtures and to real samples are still expected although monolithic stationary phases already feature high efficiency and/or high selectivity [42–44].

An excellent alternative to these approaches is the use of a dual CD system in CZE [45,46]. Enhanced PAH separations using mixtures of neutral and anionic CDs have been demonstrated in capillary [47–49] and, more recently, in microfluidic chip formats [50]. In this approach, PAHs are separated based on their different complexation constants between the neutral CD, which moves at the speed of the electroosmotic flow (EOF) and the negatively charged CD, which moves more slowly. The unique selectivity offered by the dual sulfobutyl ether-β-CD (SBE-β-CD)/methyl-β-CD (Me-β-CD) system for the analysis of the 16 US-EPA priority PAHs in contaminated soils was demonstrated [48]. However, several groups of PAHs were not fully baseline resolved (e.g. fluorene (FLR)/anthracene (ANT), chrysene (CHR)/phenanthrene (PHE)/benzo(a)anthracene (BaA), and benzo(b)fluoranthene (BbFA)/indeno(1,2,3-cd)pyrene (IP)/pyrene (Pyr)). Given these limitations, 1 year later the same group tried to introduce one more neutral CD: native γ- and α-CDs were tested [49]. Surprisingly, better PAH separation was obtained with the α-CD, which usually is not expected to interact with the biggest PAHs because of its smaller cavity size. Finally, the overall electrophoretic separation of the 16 US-EPA PAHs was enhanced by adding 4 mM α-CD to the background electrolyte (BGE), although acenaphthene (ACP)/naphthalene (NPH)/FLR and BbFA/IP were still not fully baseline resolved. Moreover, 'microprecipitation, i.e. spikes' was observed in the electropherogram and benzo(ghi)perylene (BghiP), the most hydrophobic compound, 'appeared to interact strongly with SBE-β-CD and/or the capillary wall, and in some cases it did not appear in the electropherogram', proving solubility and therefore repeatability problems [49]. Therefore, the separation of the 16 US-EPA PAHs still needed to be improved. Moreover, until now, no publication dealing with the determination of the 15+1 EU priority PAHs using CE has been published. This paper presents, to our best knowledge, the first development of CD-modified CZE with laser-induced fluorescence (LIF) detection for separation and sensitive analysis of the two lists of priority PAHs: the 16 US-EPA PAHs and the 15+1 EU priority PAHs, and its application to edible oil extracts.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene (>99.6%, BaP), BaA (>99.5%), CHR (>99.6%), BbFA (>99.5%), IP (>99.5%), benzo(j)fluoranthene (>98.5%, BjFA), 5-methylchrysene (>99.5%, MCH), dibenzo(a,l)pyrene (>99.4%, DBalP), dibenzo(a,i)pyrene (>99.9%, DBaiP), dibenzo(a,h)pyrene (>99.0%, DBahP), dibenzo(a,e)pyrene (>99.0%, DBaeP) at 10 mg/L in acetonitrile, cyclopenta(c-d)pyrene (>99.5%, CPcdP) at 100 mg/L in acetonitrile, BcFLR (>98.2%) at 10 mg/L in cyclohexane and a standard mixture of the 16 US-EPA PAHs at 10 mg/L in acetonitrile were purchased from CIL Cluzeau (Sainte-Foy-La-Grande, France). ACP (>99.0%), acenaphthylene (>99.0%, ACY), ANT (>98.0%), benzo(k)fluoranthene (>98.0%, BkFA), BghiP (>98.0%), dibenzo(ah)anthracene (>97.0%, DBahA), fluoranthene (>98.0%, FA), FLR (>98.0%), PHE (>98.0%), NPH (>99.0%), Pyr (>98.0%), and umbelliferone (≥98.0%) were supplied by Sigma-Aldrich (Saint-Quentin-Fallavier, France).

Me-β-CD with an average degree of substitution (DS) of 12.6 (average molecular weight of 1310 g/mol), urea for electrophoresis (≥99.99%) and sodium tetraborate decahydrate (≥99.5%) were from Sigma-Aldrich. SBE-β-CD with an average DS of 6.2 (average molecular weight of 2115 g/mol) was supplied by Cydex Pharmaceuticals (Lawrence, KS, USA). Methanol (MeOH), ethanol, cyclohexane, ethyl acetate, and acetonitrile (ACN) (analytical grade) were provided by VWR (Fontenay-sous-Bois, France). Ultra-pure water was delivered by a Direct-Q3 UV system (Millipore, Molsheim, France).

The final composition of the BGE was 10 mM sodium borate buffer (pH 9.2), 600 mM urea, 10 mM SBE-β-CD, 20 mM Me-β-CD in 90:10 (v/v) water–MeOH mixture. Three stock solutions of 10 mM sodium tetraborate decahydrate with 2.5 M urea, 100 mM SBE-β-CD and 100 mM Me-β-CD were prepared every week by dissolving the appropriate amounts in ultra-pure water and stored at 4 °C. The final BGE was obtained by adding 10% MeOH (v/v) and appropriate amounts of the three previous stock solutions to reach the final concentrations. All BGEs were daily prepared and filtered through 0.20 μm cellulose acetate membrane (VWR). Stock PAH mixture solutions were prepared at 1 mg/L in ACN by mixing appropriate volumes of the standard mixture of the 16 US-EPA PAHs and individual PAH standard solutions, and then stored at 4 °C. PAH standard mixtures were prepared each day by diluting the stock mixture solution in a 30:70 (v/v) MeOH–BGE mixture to the desired concentration. Stock solution of umbelliferone used as internal standard (IS) was prepared at 1.6 g/L in ethanol and diluted to 1.6 mg/L with ultra-pure water.

2.2. Apparatus and software

All CE experiments were carried out with an Agilent Technologies HP 3D system (Massy, France) hyphenated with LIF detection. Fluorescence excitation radiation was obtained from the 325 nm, 15 mW output of a HeCd laser (Model 3056-M-A02, Melles Griot, Voisins-Le-Bretonneux, France) coupled to a Zetalif Evolution LIF detector (Picometrics, Toulouse, France). All