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An experimental design based strategy to optimize a capillary electrophoresis method for the separation of 19 polycyclic aromatic hydrocarbons

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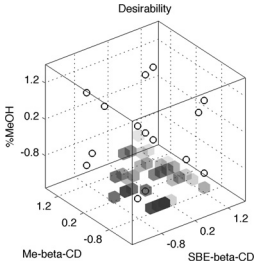
HIGHLIGHTS

- A new method for the determination of 19 PAHs in food and environment was developed.
- The full resolution of 19 PAHs in CE was reached thanks to a design of experiments.
- Cyclodextrin concentrations and methanol content were the studied factors.
- An original computational approach to deal with changes in migration order was used.
- Application to a real edible oil sample extract was successfully carried out.

GRAPHICAL ABSTRACT



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ABSTRACT

Because of their high toxicity, international regulatory institutions recommend monitoring specific polycyclic aromatic hydrocarbons (PAHs) in environmental and food samples. A fast, selective and sensitive method is therefore required for their quantitation in such complex samples. This article deals with the optimization, based on an experimental design strategy, of a cyclodextrin (CD) modified capillary zone electrophoresis separation method for the simultaneous separation of 19 PAHs listed as priority pollutants. First, using a central composite design, the normalized peak-start and peak-end times were modelled as functions of the factors that most affect PAH electrophoretic behavior: the concentrations of the anionic sulfobutylether-β-CD and neutral methyl-β-CD, and the percentage of MeOH in the background electrolyte. Then, to circumvent computational difficulties resulting from the changes in migration order likely to occur while varying experimental conditions, an original approach based on the systematic evaluation of the time intervals between all the possible pairs of peaks was used. Finally, a desirability analysis based on the smallest time interval between two consecutive peaks and on

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the overall analysis time, allowed us to achieve, for the first time in CE, full resolution of all 19 PAHs in less than 18 min. Using this optimized capillary electrophoresis method, a vegetable oil was successfully analyzed, proving its suitability for real complex sample analysis.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of important environmental and food contaminants. Many of them have been identified as mutagens, teratogens and/or carcinogens and therefore constitute a health concern [1]. PAHs are often found in complex mixtures of very similar compounds at low concentrations, requiring fast, selective and sensitive analytical methods. GC–MS [2–7] or HPLC using fluorescence detection [8–15] are the two analytical techniques most reported in the literature for the determination of PAHs in food and environmental samples. Today, CE with its high separation efficiencies, low reagent and sample consumption, and easier transfer to chip format is increasingly recognized as an interesting alternative to previous chromatographic methods [16–19]. Micellar electrokinetic chromatography (MEKC) methods with micelles formed from alkylsulfates, alkylammonium or bile salts, have been developed for the separation of these uncharged compounds [20–22]. However, because of their high hydrophobicity, PAHs tend to partition completely into the micelles and to migrate together at the same velocity as the micelles [23]. In an attempt to balance the differential partitioning of PAHs between the two pseudo-phases and therefore to enhance the resolution, modifiers are generally added to the background electrolyte (BGE), such as organic solvents or cyclodextrins (CDs). As the use of organic solvents is often unsuccessful, probably because of solvent disruption of the micelles [21,24,25], the addition of CDs appears to be the most successful strategy to improve method selectivity in MEKC. However, CD-modified MEKC still suffers from a lack of selectivity: PAHs with similar structures cannot be separated and long analysis times are generally observed [26–28].

Today, CD-modified capillary zone electrophoresis (CD-CZE) is one of the most successful strategies for PAH separation [29,30]. Recently, we developed a CD-CZE separation method using a buffer composed of a mixture of a neutral (methyl-β-CD, Me-β-CD) and an anionic CD (sulfobutyl ether-β-CD, SBE-β-CD) [31]. The separation mechanism is based on the differential distribution of PAHs between the neutral CD, traveling with the electroosmotic flow (EOF), and the negatively charged CD, traveling slower than EOF. In this study, a step-by-step approach enabled us to reach higher selectivity compared to previous published works and to significantly improve method repeatability, which was necessary to further optimize PAH separation. However, to provide a deeper insight into separation conditions and to be sure to reach the optimum selectivity, multivariate strategies were necessary. A rapid screening method of the 8 PAHs belonging to the lists established by the United States Environmental Protection Agency (US-EPA) [32] and the European Food Safety Authority (EFSA) [33] was optimized using a classical experimental design approach [34]. Indeed, as the migration order of these 8 PAHs was the same whatever the experimental condition, their fast separation was classically optimized using a desirability analysis based on the differences of peak-end and peak-start times between the seven pairs of consecutive peaks and the global analysis time only.

However, in this study dealing with the simultaneous separation of 19 PAHs, listed as priority pollutants in environmental and food samples by the US-EPA and the EFSA, plus an internal standard (IS), changes in selectivity were observed over the whole experimental domain, so that the desirability analysis could not be based on 19 migration time differences. A new optimization strategy based on the systematic evaluation of the time intervals between all possible pairs of peaks was therefore necessary. The method optimized by this approach was next applied successfully to the analysis of a real spiked oil extract, obtained from a fast, simple, and adapted solid phase extraction using molecularly imprinted polymers (MIP). It is worthy to note that this paper focuses on the qualitative point of view. The full validation step of the developed method is beyond the scope of this study and will be considered in a subsequent article.

2. Experimental section

2.1. Materials

Sodium tetraborate decahydrate (≥99.5%), Me-β-CD with a degree of substitution (DS) of 12.6 (average molecular weight of 1310 g mol^{-1}), and urea for electrophoresis (≥99.99%) were from Sigma-Aldrich (Saint-Quentin-Fallavier, France). SBE-β-CD with an average DS of 6.2 (average molecular weight of 2115 g mol^{-1}) was obtained from Cydex Pharmaceuticals (Lawrence, KS, USA). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were from VWR (Fontenay-sous-Bois, France). Ultra-pure water was delivered by a Direct-Q3 UV system (Millipore, Molsheim, France). Benz[*a*]anthracene (>99.5%, BaA), benzo[*b*]fluoranthene (>99.5%, BbFA), benzo[*j*]fluoranthene (>98.5%, BjFA), benzo[*a*]pyrene (>99.6%, BaP), chrysene (>99.6%, CHR), dibenzo[*a,e*]pyrene (>99.0%, DBaeP), dibenzo[*a,h*]pyrene (>99.0%, DBahP), dibenzo[*a,i*]pyrene (>99.9%, DBaiP), dibenzo[*a,l*]pyrene (>99.4%, DBalP), indeno[*1,2,3-cd*]pyrene (>99.5%, IP), 5-methylchrysene (>99.5%, MCH) at 10 mg L^{-1} in ACN, cyclopenta[*c-d*]pyrene (>99.5%, CPcdP) at 100 mg L^{-1} in ACN, and benzo[*c*]fluorene (>98.2%, BcFLR) at 10 mg L^{-1} in cyclohexane were supplied by CIL Cluzeau (Sainte-Foy-la-Grande, France). Acenaphthene (>99.0%, ACP), acenaphthylene (>99.0%, ACY), anthracene (>98.0%, ANT), benzo[*k*]fluoranthene (>98.0%, BkFA), benzo[*ghi*]perylene (>98.0%, BghiP), dibenz[*a,h*]anthracene (>97.0%, DBahA), fluoranthene (>98.0%, FA), fluorene (>98.0%, FLR), naphthalene (>99.0%, NPH), phenanthrene (>98.0%, PHE), pyrene (>98.0%, Pyr), and umbelliferone (≥98.0%) used as IS were purchased from Sigma-Aldrich. Stock solutions at 100 mg L^{-1} of these PAHs were prepared by dissolving in ACN.

Electrolytes were daily prepared by mixing appropriate volumes of sodium tetraborate decahydrate, urea, Me-β-CD, and SBE-β-CD stock solutions with MeOH as described previously [34], and filtered through a 0.20 μm cellulose acetate membrane (VWR). Stock solution of umbelliferone was prepared at 1.6 g L^{-1} in ethanol and diluted to 16.0 mg L^{-1} in water.

PAHs studied were those belonging to both US-EPA and EFSA lists each gathering 16 PAHs. As 8 PAHs are in common between these two priority lists, 24 PAHs in all are currently under regulation. When using LIF detection with excitation wavelength at 325 nm, only 20 out of the 24 compounds could be detected. Indeed, NPH, ACY, CPcdP, and FLR did not give measurable fluorescence signal even at 10 mg L^{-1}. BcFLR was only available in cyclohexane and thus could not be added to the working standard mixture of the other PAHs without inducing the formation of a microemulsion. For these reasons, these five compounds were not studied here. Two stock mixture solutions were prepared in ACN by mixing appropriate volumes of PAH standard solutions and IS stock