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

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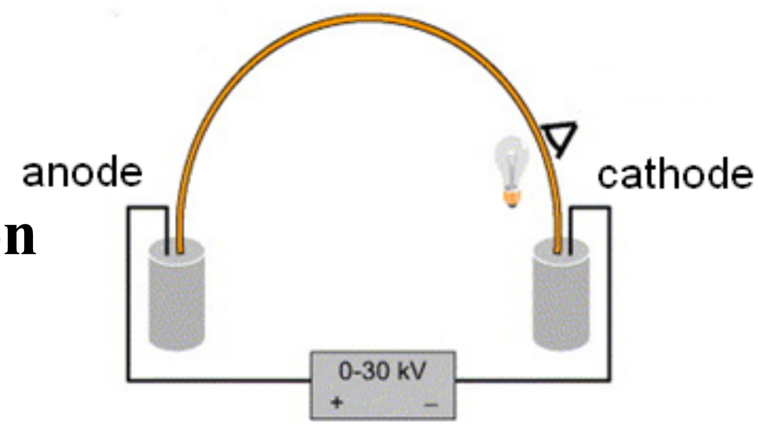
Introduction

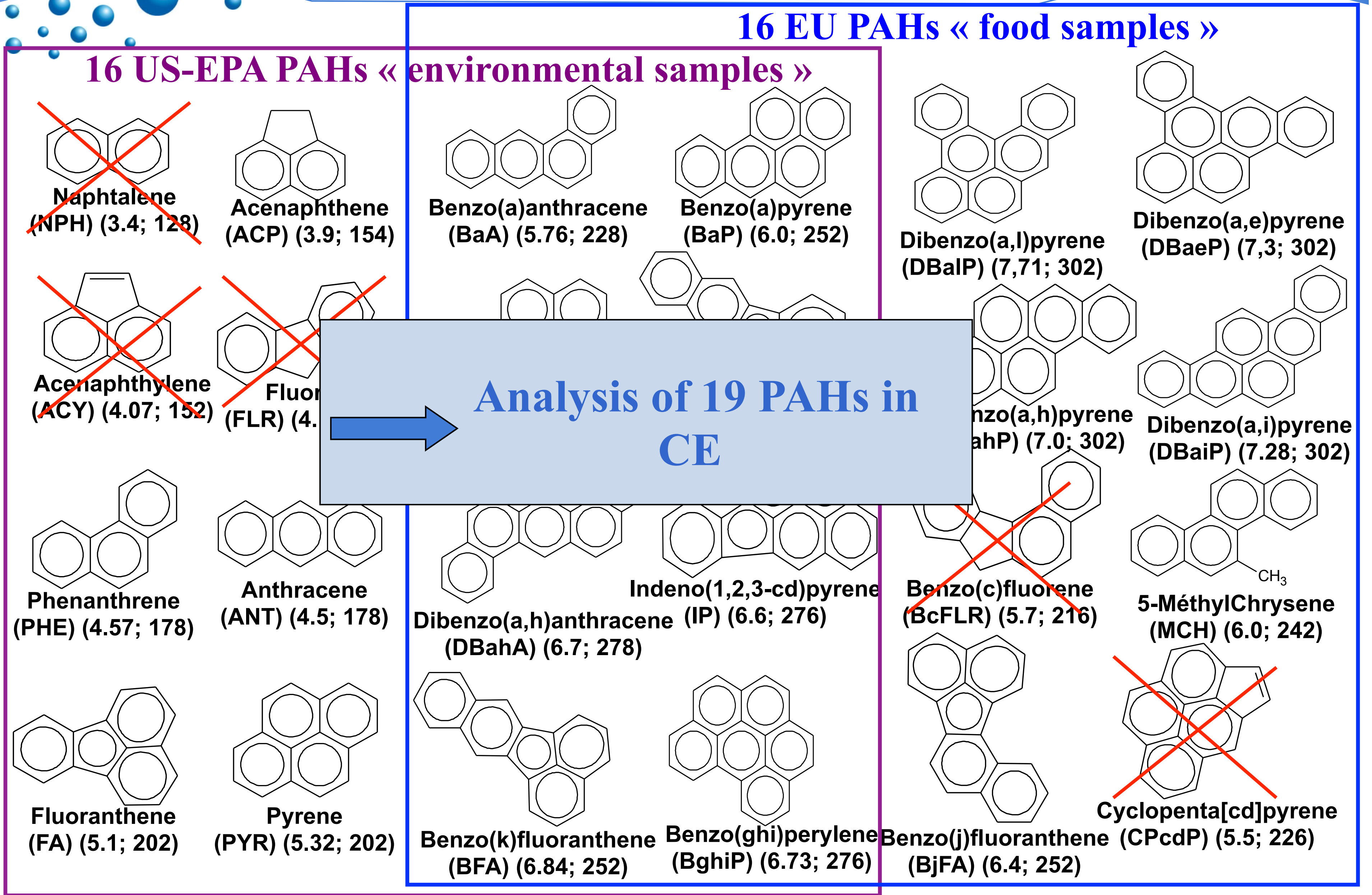
Research project:

➢ **development and optimisation of PAH analysis in CE using Laser-Induced Fluorescence detection (LIF)**

☺ **High efficiencies**
Low operational costs
Low reagent and sample consumption
Automated analysis
Sensitivity

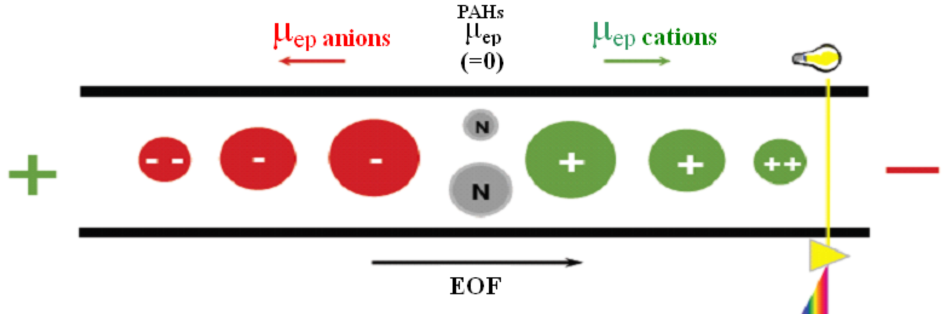


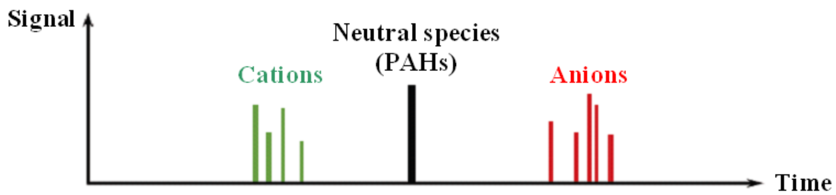
Introduction: targeted compounds



Introduction: separation strategy

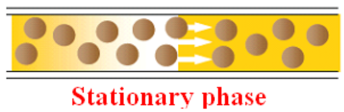
CZE: analysis of water-soluble and charged species



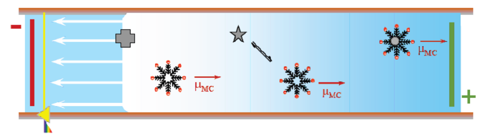


Analysis of PAHs in CE: different strategies

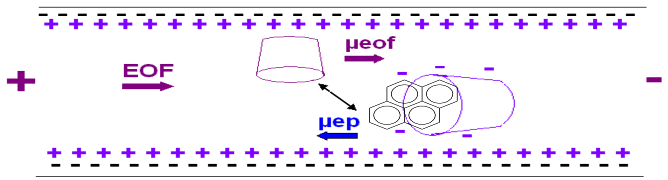
Electrochromatography



Micellar Electrokinetic Chromatography



Cyclodextrin-modified CZE



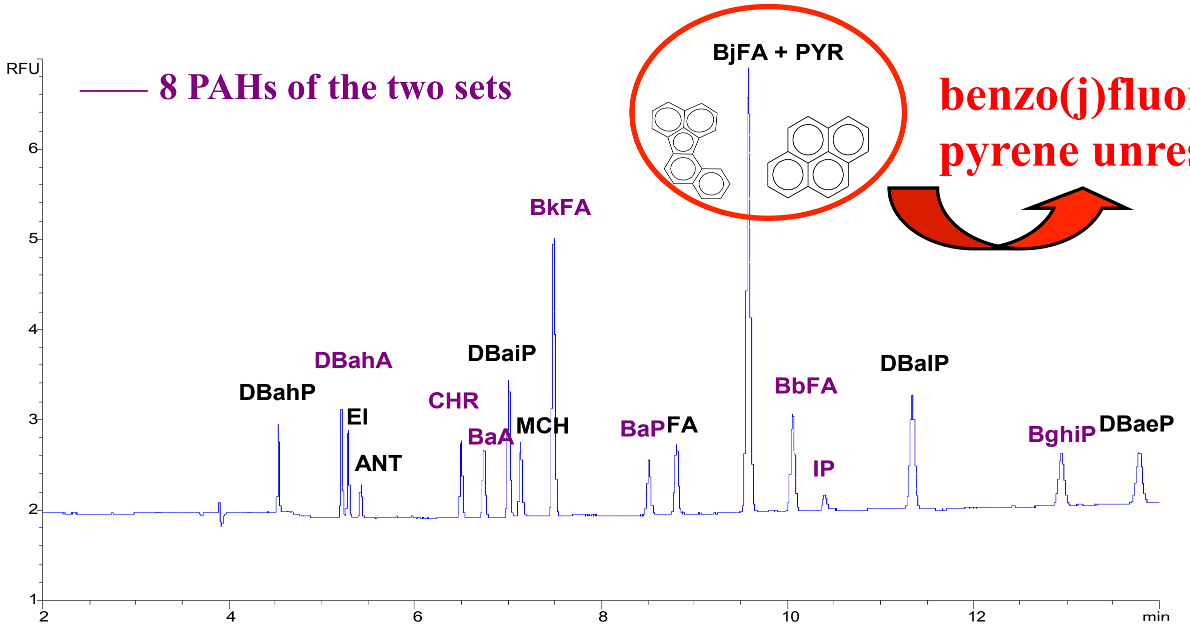
Introduction: CD-CZE

CD-CZE analysis of PAHs:

- **Dual CD system: 1 neutral CD (Me-β-CD) + 1 anionic CD (SBE-β-CD)**
- **Separation mecanism: PAHs differential partitioning between the two CDs**

→ **high selectivity**

- **Best results found with an univariate method (one-variable-at-a-time):**
  - **10 mM sodium borate buffer (pH 9.2), 600 mM urea, 10 mM SBE-β-CD, 20 mM Me-β-CD in 9:1 (v/v) water-methanol mixture**
  - **electrophoretic separation of 16 PAHs in 15 min with R_s> 1.8**



benzo(j)fluoranthene and pyrene unresolved

COST versus multivariate approaches

COST approach: Change One Separate factor at a Time

☹

High number of experiments

Local and not global optimum

Interactions not studied

Isolated experiments

No mapping of experimental domain

Design of experiments
How? Simultaneous variations of factors (matrix of experiments)

☺

Determination of factors effects and interactions on responses

Response surfaces graphically represented

Global optimum achieved

Use of a design of experiments to optimize the selectivity of the analysis of PAHs in CD-CZE

Choice of the factors

Identification of the factors most influencing the PAH electrophoretic behavior:

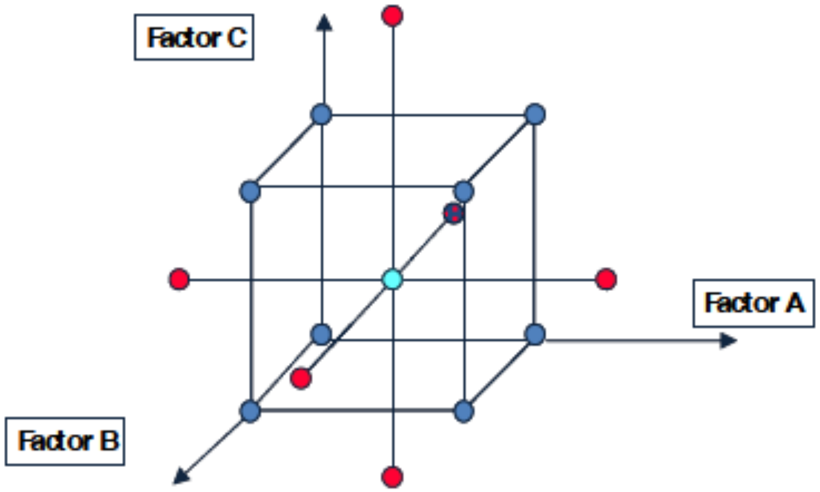
- **[SBE-β-CD]**
- **[Me-β-CD]**
- **% MeOH**

} **Impact on the selectivity, BGE viscosity and ionic strength (for [SBE-β-CD] only), and PAH solubility**

Factors maintained constant:

- **[borate] = 10 mM and [urea] = 600 mM (high concentration to assure PAH solubility) in BGE**
- **Temperature 25°C and ΔV = 14 kV (no joule effect)**
- **Bare fused-silica capillaries 50 µm I.D. x 49 cm (LIF detection at 33.5 cm)**

Central composite design with 3 factors:



Combination of:

- one two-level full factorial design (8 vertice points)
- a star design (6 axis points)
- 6 center points to evaluate experimental error

20 experiments

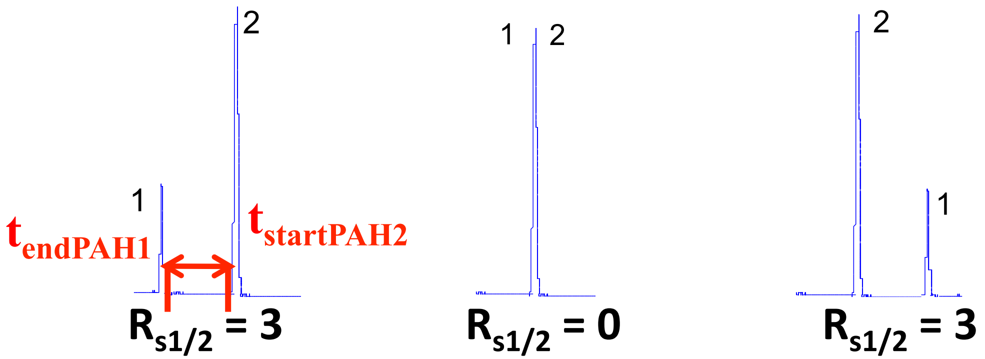
Variation domains of the factors

Determined from preliminary experiments:

- **5 mM ≤ [SBE-β-CD] ≤ 15 mM**
  - **5 mM: to assure enough selectivity and preserve PAH solubility in sample & BGE for (-1,-1,-1), (-1,6,0,0) and (0,0,-1,6)**
  - **15 mM: to keep analysis time < 45 min for (+1,6,0,0), (0,0,+1,6) and (+1,-1,+1)**
- **5 mM ≤ [Me-β-CD] ≤ 40 mM**
  - **5 mM: to preserve PAH solubility in sample & BGE**
  - **40 mM: to limit BGE viscosity**
- **10 % ≤ % MeOH ≤ 25 %**
  - **10 %: to avoid repeatability problems due to possible evaporation, and preserve PAH solubility in sample & BGE for (0,0,-1,6), (-1,-1,-1) and (-1,6,0,0)**
  - **25 %: to prevent CD precipitation for (0,0,+1,6) and (+1,+1,+1) and keep analysis time < 45 min for (+1,6,0,0), (0,0,+1,6) and (+1,-1,+1)**

Choice of the responses

- **To study PAH electrophoretic behavior:**
  - **migration times normalized by the EOF migration time**
- **To study the quality of the separation (selectivity):**
  - **inversion in selectivity:**



  - **~~resolution~~: differences of normalized migration times between peak start and peak end of two consecutive peaks ($t_{startPAH2}$-$t_{endPAH1}$)**
- **To study analysis time:**
  - **migration time of the last peak**

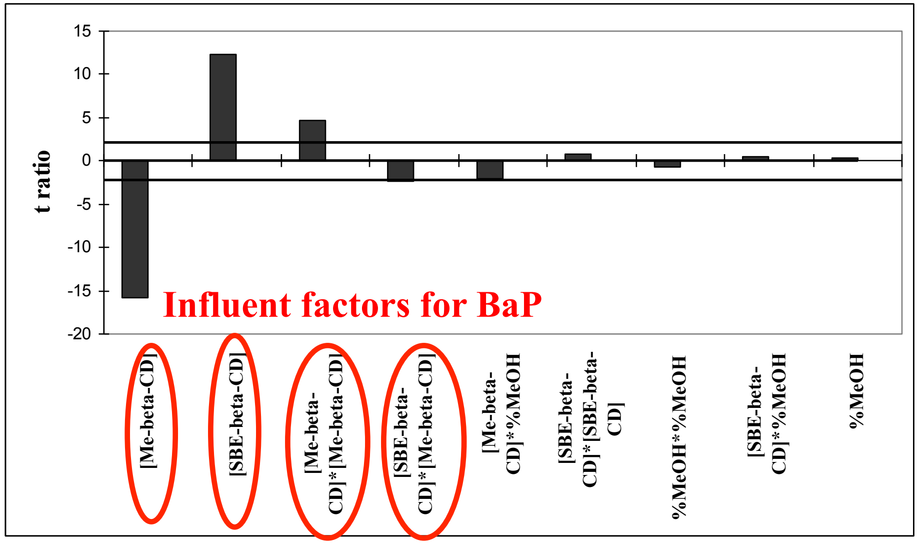
Effects of factors on PAH migration (1/2)

- **Second-order polynomial quadratic model (response surface)**

$$\eta\,(X_{1 \leq i \leq 3}) = \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=2}^{3} \sum_{j=1}^{i-1} \beta_{ij} X_i X_j$$

Predicted response — **Factors** — **Main coefficients** — **First order interaction coefficients** — **Quadratic coefficients**

- **Estimation of model coefficients for PAH normalized migration times**
  - **To identify factors most influencing PAH electrophoretic behavior**



Regression coefficients plot representing main, nonlinear, and interaction effects of factors on BaP normalized migration time.

BaP normalized migration time

Factors	Regression coefficients	t ratio	Prob >|t|
[Me-β-CD]	-0.553	-15.79	<,0001
[SBE-β-CD]	0.429	12.24	<,0001
[Me-β-CD]²	0.167	4.63	0.0009
[SBE-β-CD] x [Me-β-CD]	-0.103	-2.30	0.0439
[Me-β-CD] x %MeOH	-0.089	-1.98	0.0764
[SBE-β-CD] x [SBE-β-CD]	0.028	0.79	0.4487
%MeOH x %MeOH	-0.027	-0.74	0.4781
[SBE-β-CD] x %MeOH	0.022	0.50	0.6302
%MeOH	0.014	0.40	0.6980

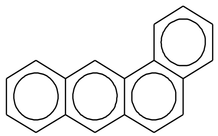
Effects of factors on PAH migration (2/2)

- Same work on other PAH normalized migration times:
  - significant:
    - [SBE-β-CD]: positive effect
    - [Me-β-CD]: negative effect
    - $[Me\text{-}\beta\text{-}CD]^2$ ⟶ choice of a second-order polynomial quadratic modeling relevant
  - sometimes significant:
    - %MeOH (3/19)
    - [SBE-β-CD] x [Me-β-CD] (4/19)
    - [Me-β-CD] x %MeOH (4/19)
  - never significant:
    - $[SBE\text{-}\beta\text{-}CD]^2$
    - $\%MeOH^2$
    - [SBE-β-CD] x %MeOH

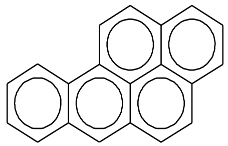
DOE data exploitation: two objectives (1/2)

- **Separation optimization of two PAH mixtures in CE:**

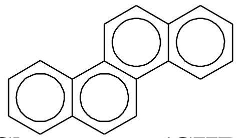
  1) 8 PAHs of food and environmental interest since belonging to the 2 lists of pollutants (US-EPA + EFSA)



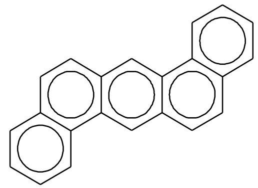
Benzo(a)anthracene (BaA) (5.76; 228)



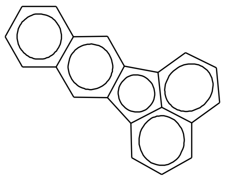
Benzo(a)pyrene (BaP) (6.0; 252)



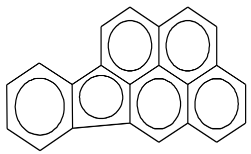
Chrysene (CHR) (5.7; 228)



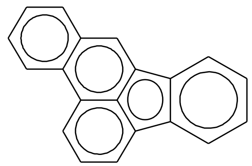
Dibenzo(a,h)anthracene (DBahA) (6.7; 278)



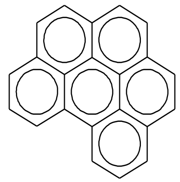
Benzo(k)fluoranthene (BFA) (6.84; 252)



Indeno(1,2,3-cd)pyrene (IP) (6.6; 276)



Benzo(b)fluoranthene (BbFA) (6.57; 252)



Benzo(ghi)perylene (BghiP) (6.73; 276)

- **No change in migration order → classical optimization strategy: response surface methodology using desirability analysis with JMP software**

See poster n°27?

DOE data exploitation: two objectives (2/2)

2) 19 PAHs currently under regulation → demonstration of the selectivity of the method (PAHs can be found as interferents in complexe mixtures)

- **Reversal in migration order → new optimization strategy: systematic approach using Matlab software**
- **Second-order polynomial quadratic model → response surfaces of normalized migration times of peak start and peak end of all 19 peaks**

$$\eta(X_{1 \leq i \leq 3}) = \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=2}^{3} \sum_{j=1}^{i-1} \beta_{ij} X_i X_j$$

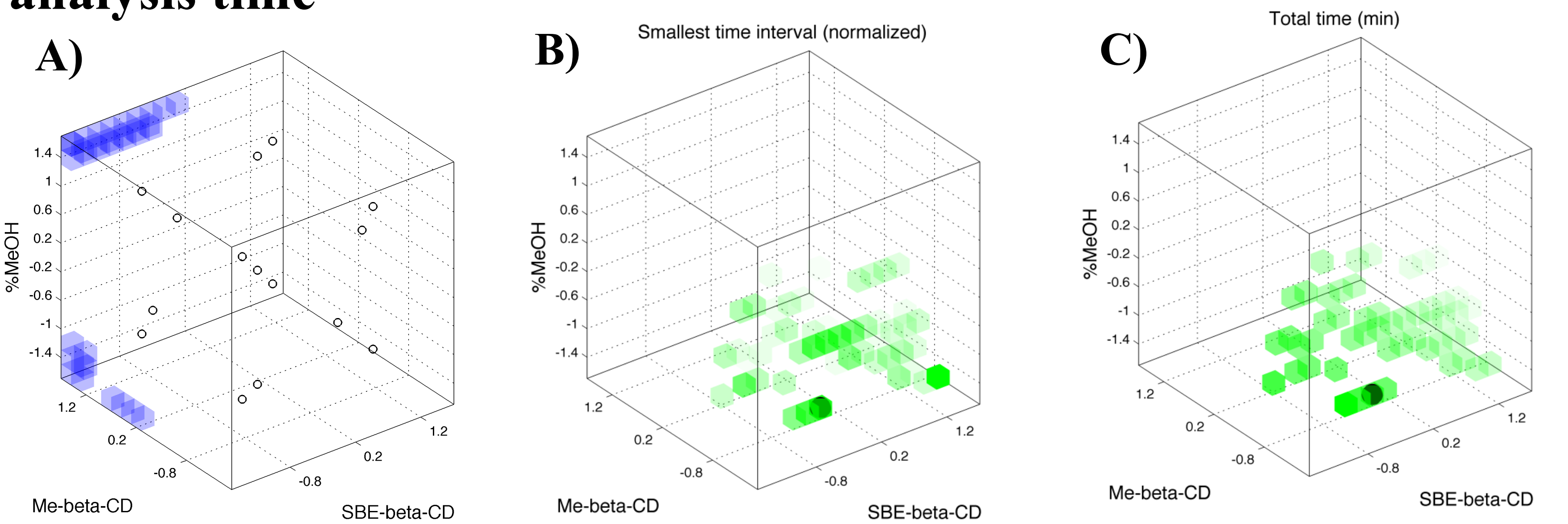
19 PAHs: separation optimization (1/2)

- 3D experimental domain (cube side: -1.6;1.6): division in 17x17x17 cubes → 4913 conditions

- Differences of normalized migration times between all the couples of peaks were computed → cubes corresponding to overlapping peaks discarded: 171 conditions for which $t_{startPAHj}/teo - t_{endPAHi}/teo > 0$

19 PAHs: separation optimization (2/2)

- **For retained experimental conditions: desirability analysis on minimum interval between two consecutive peaks and global analysis time**



Representation of experimental points and those predicted by the model

A) Predicted absurd points in blue and DOE experimental points in white

B) Predicted smallest interval for retained conditions: optimum points in dark green

C) Predicted analysis time for retained conditions: optimum points in dark green

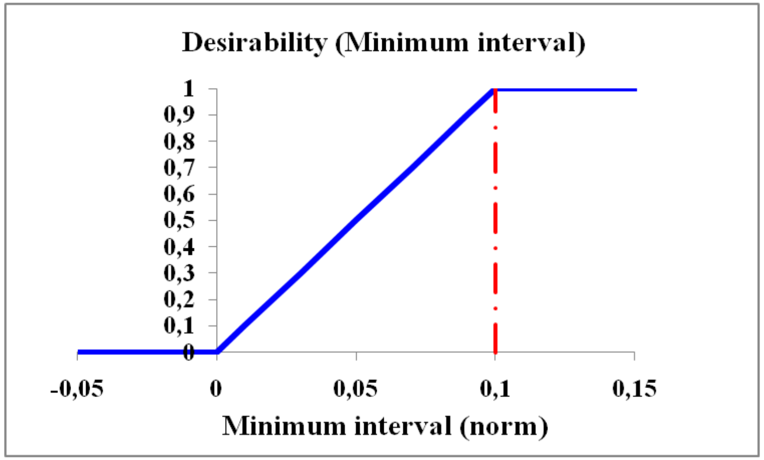
Desirability analysis

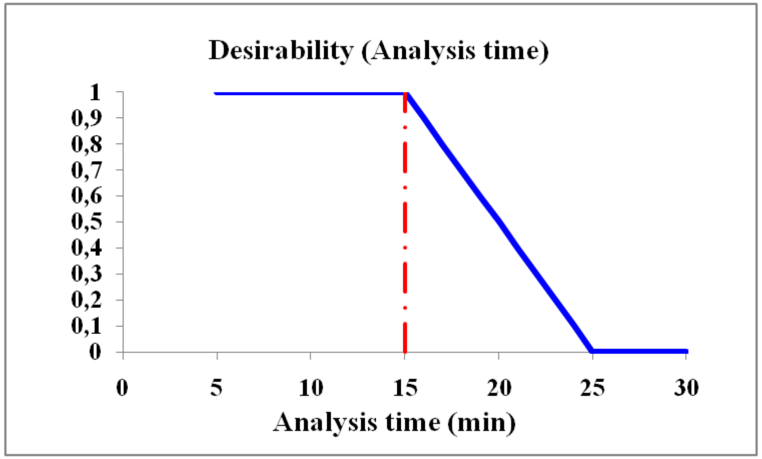
- **Aim: maximum PAH separation in a minimum analysis time**

Multicriteria optimization → Use of desirability functions

- **Desirability functions: each response transformed on a scale between 0 (the most undesirable outcome) and 1(the most desirable situation)**

- **di: individual desirability (one for each response)**

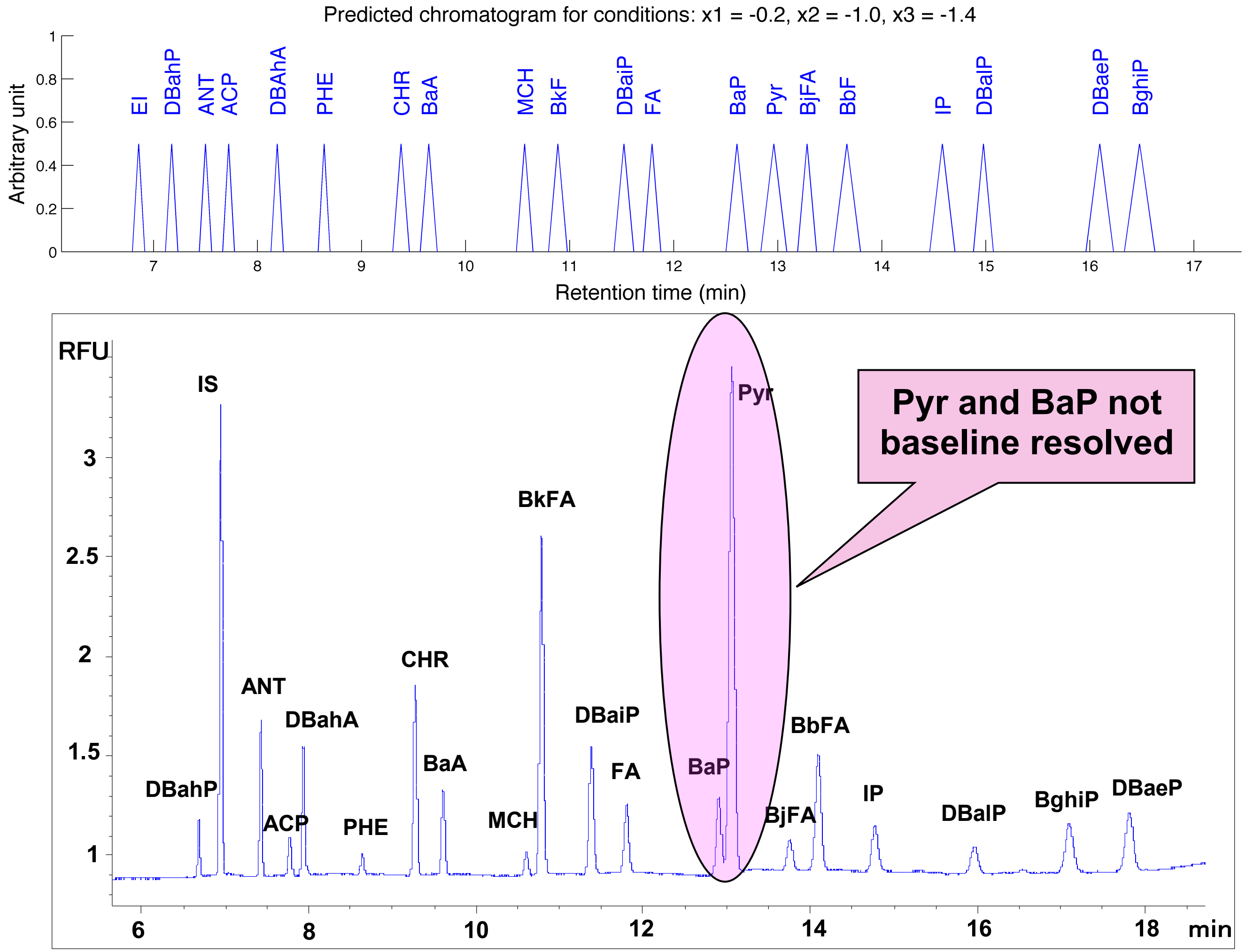




- **Global desirability:** $D = \prod_{i=1}^{n} d_i^{\,p}$ **p: power = 1**

Predicted optimum: (-0.2;-1;-1.4) ➡ 9.4 mM SBE-β-CD; 12.0 mM Me-β-CD and 11.2 % MeOH

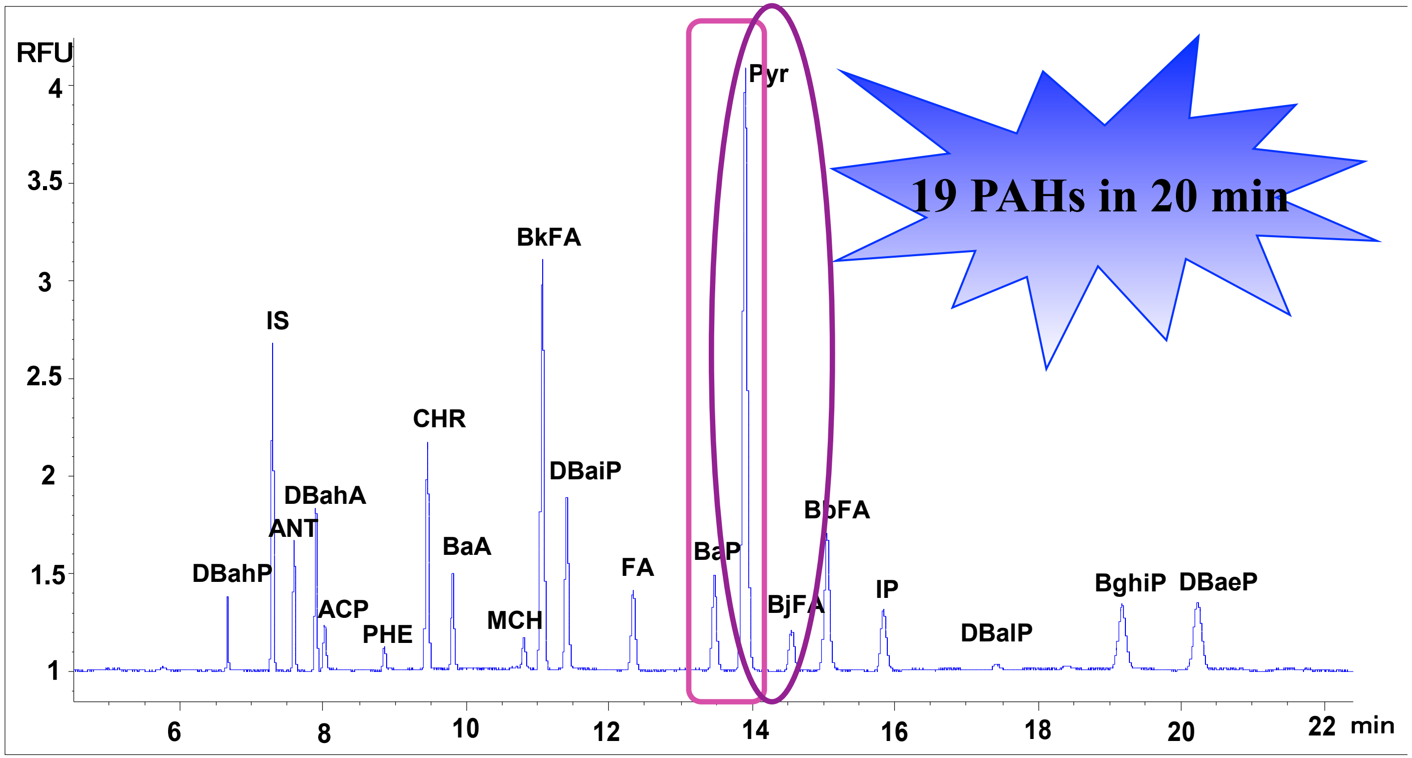
Experimental Validation



Bare fused-silica capillary, 50 µm I.D. x 49 cm (LIF detection at 33.5 cm). BGE: 10 mM sodium borate buffer (pH 9.2), 600 mM urea, 9.4 mM SBE-β-CD, and 12.0 mM Me-β-CD in 88.8 % /11.2 % (v/v) water-MeOH mixture. Temperature, 25°C. Applied voltage, 14 kV. Excitation: 325 nm, emission: 350 nm. Sample: PAHs at about 100 µg/L in 10 % ACN / 90 % electrolyte. IS: umbelliferone

Tests around the predicted optimum

- Predicted optimum: (-0.2;-1;-1.4) ➡ 9.4 mM SBE-β-CD; 12.0 mM Me-β-CD and 11.2 % MeOH
- Best conditions found: (-0,2; -0,8; -1,4) ➡ 9.4 mM SBE-β-CD; 14.1 mM Me-β-CD and 11.2 % MeOH



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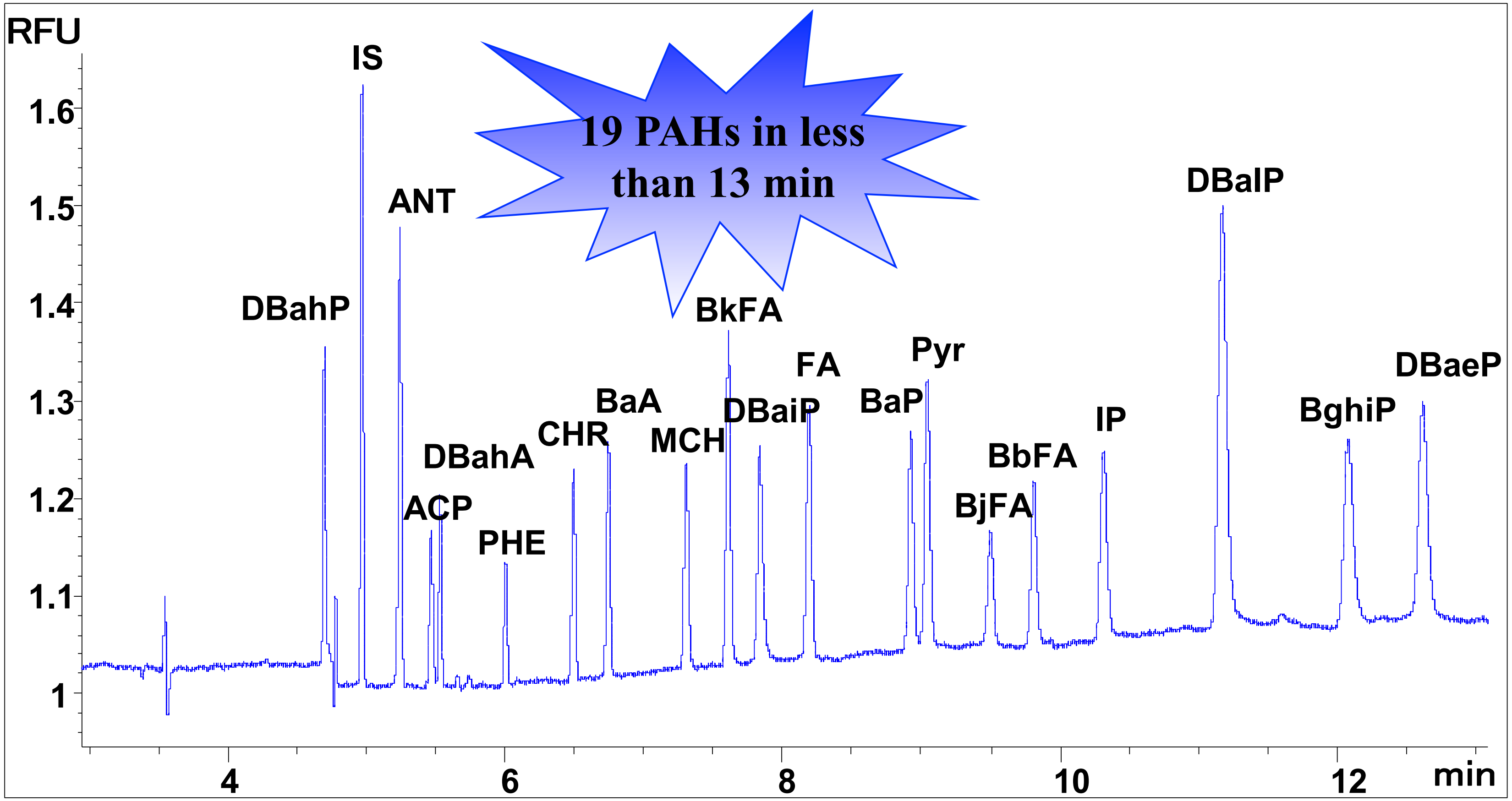
Voltage optimization

- Test of Joule effect ➡ inscrease in voltage from 14 kV to 17 kV ➡ decrease in analysis time from 20 min to 13 min



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Conclusion

- **2 CE methods for the separation of PAHs were optimized using a design of experiments**
  - **The most studied 8 PAHs baseline resolved in less than 8 min**
  - **19 PAHs under regulation baseline resolved in less than 13 min with Rs > 1.3**
- **Experimental designs: efficient tool for the optimization of separation methods**
- **Futur objectives**
  - **Robustness evaluation using another multivariate approach (screening designs)**
  - **Quantitative validation on real food samples (edible oils)**

Thank you for your attention.

Questions?