

Color encoded microbeads-based flow cytometric immunoassay for Polycyclic Aromatic Hydrocarbons in food.

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Objectives

Development of a highly sensitive flow cytometry-based immunoassay (FCIA), with multiplex capabilities, to detect several PAHs in food (fish and cereals) using benzo[a]pyrene (BaP) as a marker.

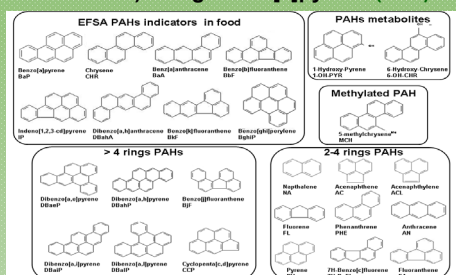


Figure 1: Molecular structures of the 26 food-related PAHs used during the evaluation of the previously developed ELISA* and/or the presently described FCIA

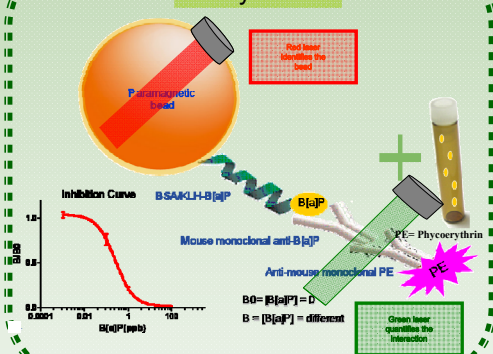
* D. Matschulat, A. Deng, R. Niessner, D. Knopp, *The Analyst*, 130 (2005) 1078.

Conclusions

- ✓ The FCIA is a highly sensitive assay for BaP with an IC₅₀ of 0.3 µg L⁻¹ similar to that obtained in a previously developed ELISA in buffer using the same antibodies.
- ✓ CHR and IP, two other EFSA indicators for PAH contamination in food are also detected by this FCIA (with cross-reactivities (CRs) of 81 and 53%, respectively).
- ✓ The developed FCIA was shown to be applicable to real food extract analysis, such as smoked carp and wheat flour extract. Next target will be to simplify the sample preparation.
- ✓ The FCIA offers a better alternative to existing screening methods and can be extended for multi-analyte food profiling.

Assay format and Results

Assay format



Antibody evaluation

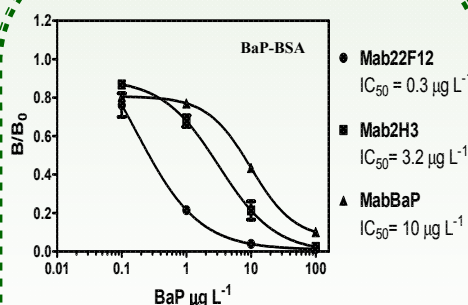


Figure 2: BaP calibration curves obtained in the FCIA with the mouse monoclonal antibodies (Mab) against BaP Mab22F12, Mab2H3 and MabMaBP in combination with BaP protein conjugates coated beads BaP-BSA in which B_0 is the maximum MFI of the blank measurement and B the MFI obtained with the different BaP concentrations. Each point represents the mean of three replicates $\pm$ SD

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26- food related PAHs CR%

Table 1: Percentages of cross-reactivity (CR%) obtained with Mab22F12 and Mab2H3 for the 26 food-related PAHs in the formerly developed ELISA and/or in the presently described FCIA. The PAHs marked with the * belong to the group of PAH8 according to EFSA's opinion.

	CR (%)			
	Mah22F12		Mah2H3	
	FCIA	ELISA	FCIA	ELISA
BaP*	100	100	100	100
CHR*	53	77	24	63
BaA*	7	13	30	149
BbF*	8	24	23	101
BkF*	4	5	6	50
IP*	25	45	81	76
BghiP*	0	1	0	2
DBahA*	0	0	6	38
DBacP	0	-	0	-
DBahP	1	-	0	-
DBaiP	1	-	10	-
DBalP	0	-	0	-
NA	0	0	0	0
ACL	10	0	27	0
AC	25	0	27	0
FL	0	0	5	1
PHE	0	1	0	9
AN	3	1	0.3	4
FA	0	0	0	0
PY	1	18	0	30
MCH	11	-	30	-
6-OH-CHR	2	-	0	-
1-OH-PYR	2	-	0.1	-
BjF	146	-	127	-
CCP	1	-	16	-
7H-BcFL	9	-	37	-

Food sample extracts measured with the two ECIAs

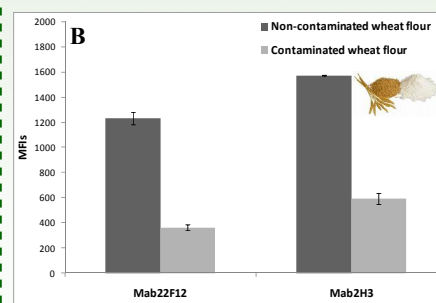
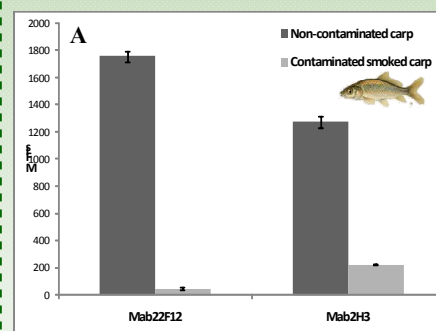


Figure 3: Responses (MFIs) obtained with non-contaminated (black columns) and with PAHs contaminated extracts (grey columns) of (A) carp and (B) wheat flour measured in the FCIA with both Mabs (Mab22F12 and Mab2H3).