中国农业科学院油料作物研究所
OILCROPS RESEARCH INSTITUTE,CHINESE ACADEMY OF AGRICULTURAL SCIENCES

Congratulations to 16th International Rapeseed Congress/IRC 2023

A rapid low-cost and easy method based on time resolved fluorescence lateral flow immunoassay for benzo(a)pyrene in rapeseed oil

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Chinese Academy of Agricultural Sciences, Wuhan, China

27nd September, 2023 SYDNEY

Outlines

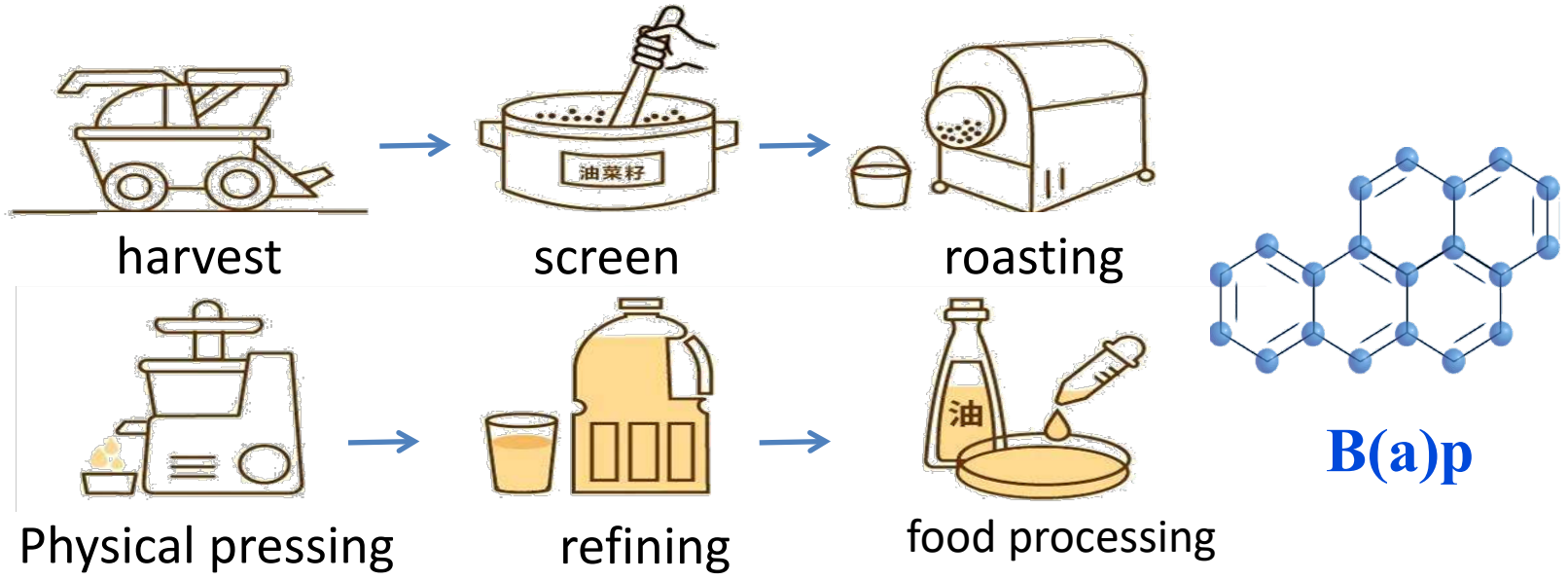
- The contamination of benzo(a)pyrene in rapeseed oil
- Development of the anti-B[a]p monoclonal antibody
- Establishment of time resolved fluorescence lateral flow immunoassay
- Conclusion

The contamination of B(a)p in rapeseed oil

The contamination of B(a)p in rapeseed oil

- B[a]P is a kind of polycyclic aromatic hydrocarbons (PAHs)
- Widely in the atmosphere, water and foods as contaminants
- Incomplete combustion/pyrolysis of organic matters

  1. Industrial processes (for drying oil seeds prior to oil extraction)
  2. Food processing (i.e. dry, roasting, smoking, babecuing)



B(a)p

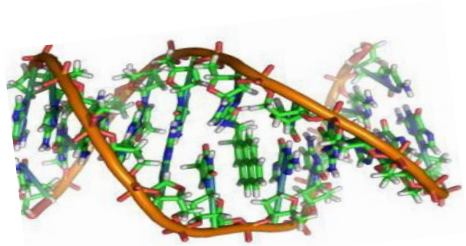
$C_{20}H_{12}$, 252.309

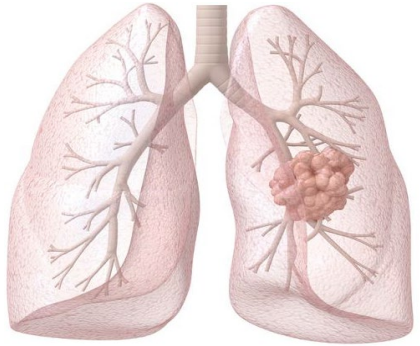
- fat soluble
- insoluble in water

Threaten in Public Health

WHO IARC Group 1 carcinogen

- Strong carcinogenicity
- Mutagenic and teratogenic effects
- Potentially fatal chronic toxicity





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David H. Phillips

Nature **303**, 468–472 (1983) | Cite this article

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It is fifty years since the publication of the report on th identification of the potent chemical carcinogen benzo of over 10 years of research instigated and directed by leading to that discovery are of interest in themselves. unravelling the metabolic fate of polycyclic aromatic h to our understanding of the mechanism of chemical ca

Cell

Volume 32, Issue 1, January 1983, Pages 239-246

Article

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Vincent L. Wilson *, Peter A. Jones

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Science

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HOME › SCIENCE › VOL. 274, NO. 5286 › PREFERENTIAL FORMATION OF BENZO[A]PYRENE ADDUCTS AT LUNG CANCER MU

REPORT

Preferential Formation of Benzo[a]pyrene Adducts at L Mutational Hotspots in *P53*

MIKHAIL F. DENISSENKO, ANNIE PAO, MOON-SHONG TANG, AND GERD P. PFEIFER Authors Info & Affiliations

SCIENCE · 18 Oct 1996 · Vol 274, Issue 5286 · pp. 430-432 · DOI: 10.1126/science.274.5286.430

196 5

Abstract

Cigarette smoke carcinogens such as benzo[*a*]pyrene are implicated in the development o cancer. The distribution of benzo[*a*]pyrene diol epoxide (BPDE) adducts along exons of the in BPDE-treated HeLa cells and bronchial epithelial cells was mapped at nucleotide resolu Strong and selective adduct formation occurred at guanine positions in codons 157, 248, a These same positions are the major mutational hotspots in human lung cancers. Thus, tar adduct formation rather than phenotypic selection appears to shape the *P53* mutational s lung cancer. These results provide a direct etiological link between a defined chemical car human cancer.

THE LANCET

Volume 349, Issue 9052, 1 March 1997, Page 652

Correspondence

Exposure to benzo[a]pyrene

ML Williams [a], RL Maynard [a]

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References (1)

G Myddelton

Maximum Limit of B[a]P

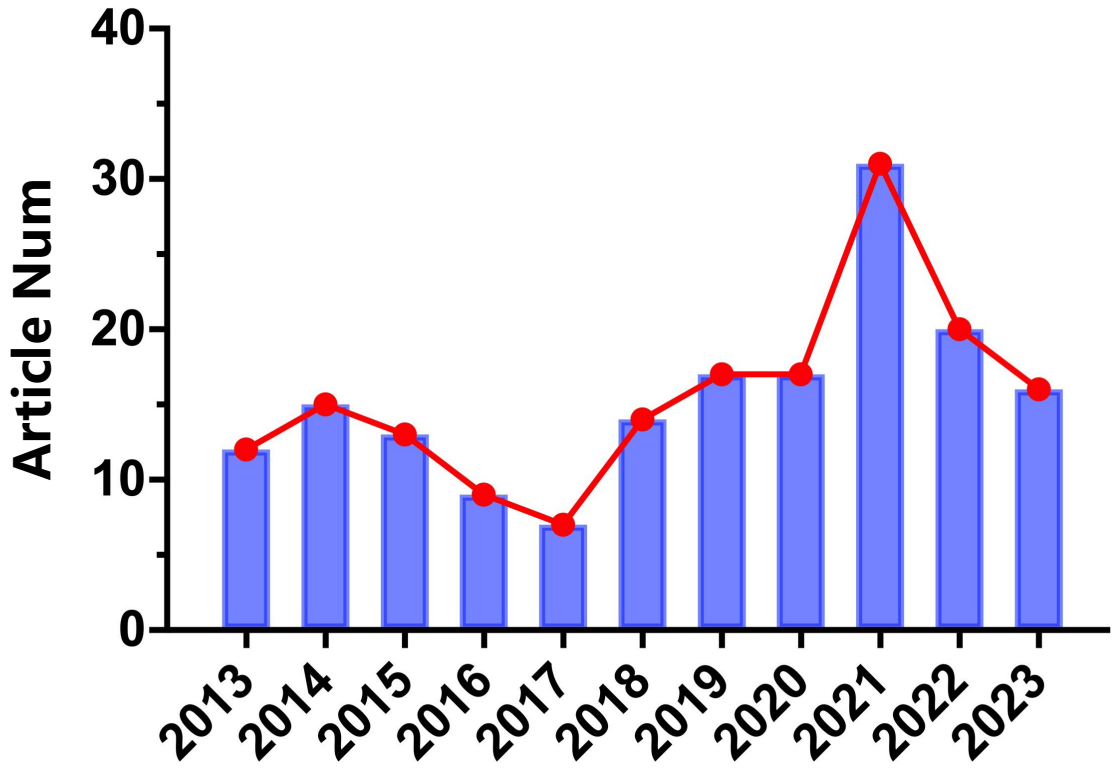
Countries/organization	Products	Limit of B[a]P
GB 2762-2022 （China）	cereals and their products	2.0 μg/kg
NY/T 751-2021 （China）	rapeseed oil	5.0 μg/kg
(EU) No 835-2011	edible oil	2.0 μg/kg
Codex Alimentarius Commission (CAC)	edible oil	5.0 μg/kg
U.S. Food and Drug Administration (FDA)	food and feed	50 μg/kg
Australian Food Standard	cooked meat	10 μg/kg
World Health Organization (WHO)	water	0.2 μg/L

ISO/National Standard Method for B[a]P/PAH analysis

- GB 5009.27-2016, HPLC, Determination of Benzo[a]pyrene in Foods, China
- KJ 201910, Colloidal gold immunochromatography, edible oil, China
- ISO 15302:2017, HPLC, Animal and vegetable fats and oils — Determination of benzo[a]pyrene-Reverse-phase high performance liquid chromatography method
- SW-846 Test Method 8310, HPLC, U.S. Environmental Protection Agency
- FDA food program compendium analytical laboratory methods: NO. C-002.01 Screening and Determination of Polycyclic Aromatic Hydrocarbons in Seafoods Using QuEChERS-Based Extraction and High-Performance Liquid Chromatography with Fluorescence Detection

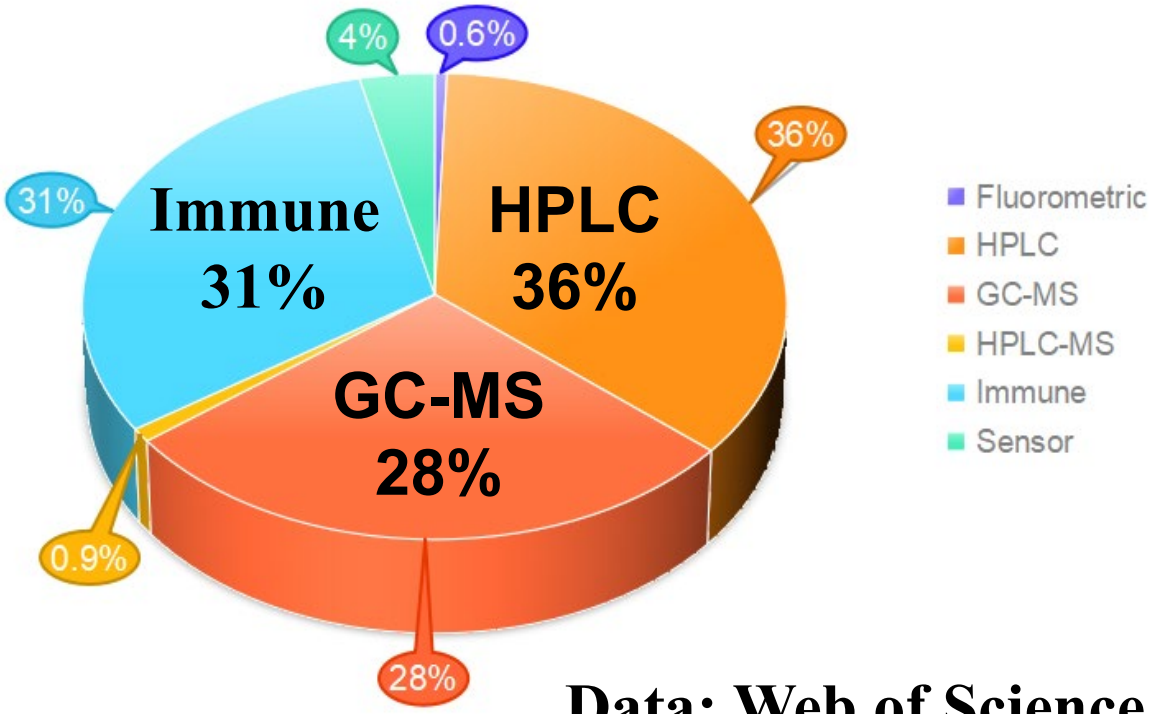
Publications for B[a]P analysis

Total numbers of article per year from 2013-2023



Immunoassay methods: 1456

Conventional methods: 3086



Data: Web of Science

Determination of the level of B[a]P

- **Conventional methods: HPLC、GC-MS、FA**

  relies on large instruments, professional operaters

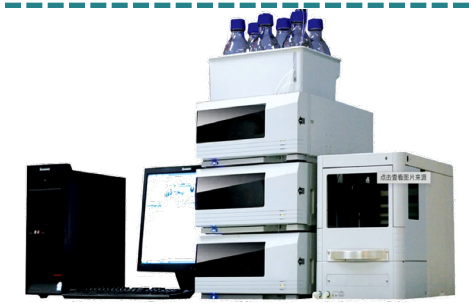
- **Immunoassay methods: ELISA、immunochromatographic strip**

  it is based on the recognition of B[a]P by specific antibodies

  high sensitivity, good specificity, rapid and simple

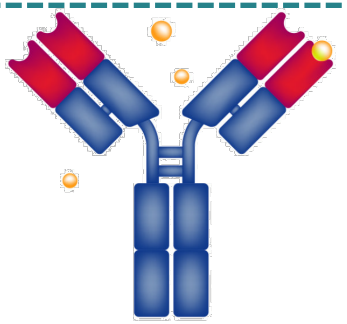
High-quality antibodies are the key factors for high sensitivity detection

Fluorescence spectrophotometers



HPLC

GC-MS

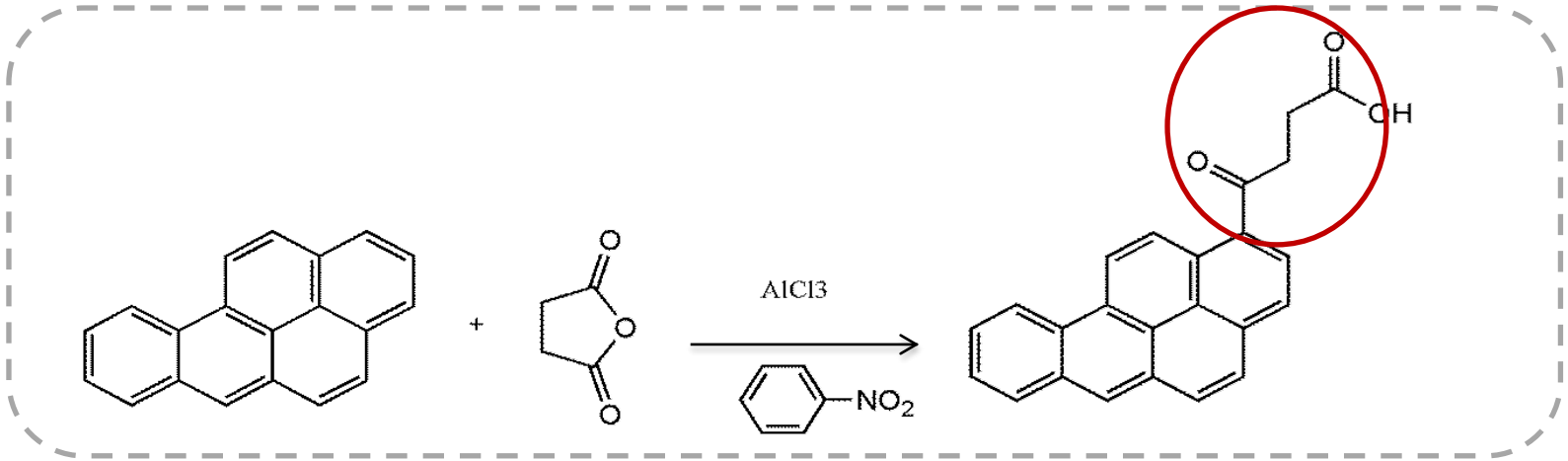


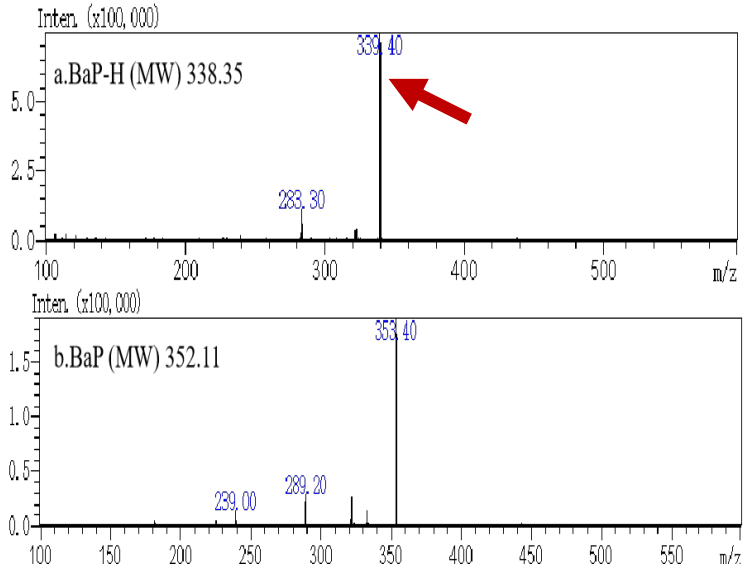
immunodetetion

Development of the anti-B[a]P mAb

Systhesis and Identification of B[a]P hapten

- The hapten was successfully produced from B[a]P via Friedel - Crafts acylation reaction, and was characterized by LC-MS
- The MS calculations for hapten were 338.35, found 339.40
- The hapten have a 4-C spacer arm, which is important for its immunogenicity



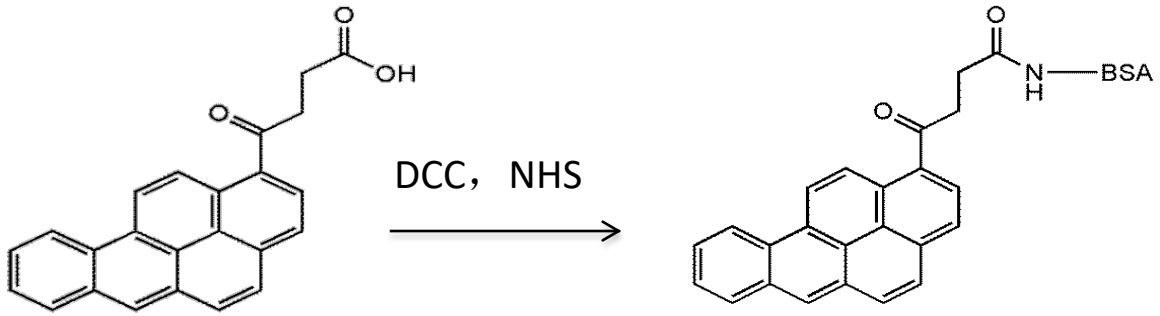


Systhesis and Identification of antigens

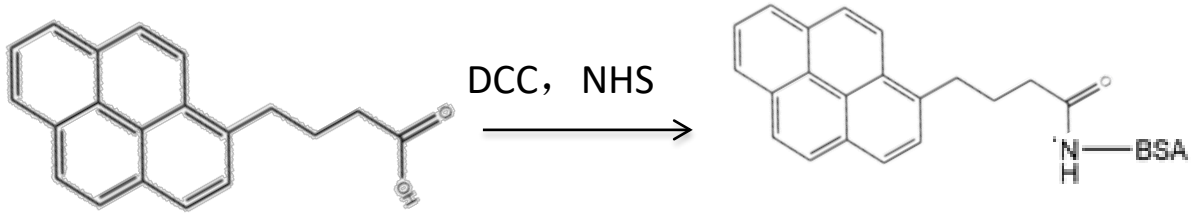
I. The B[a]P-BSA and PyBA-BSA were characterized by UV-Vis spectrophotometry

II. The peaks became significantly smooth and red shifted at 258-290nm and 300-360nm with hapten and the corresponding antigens

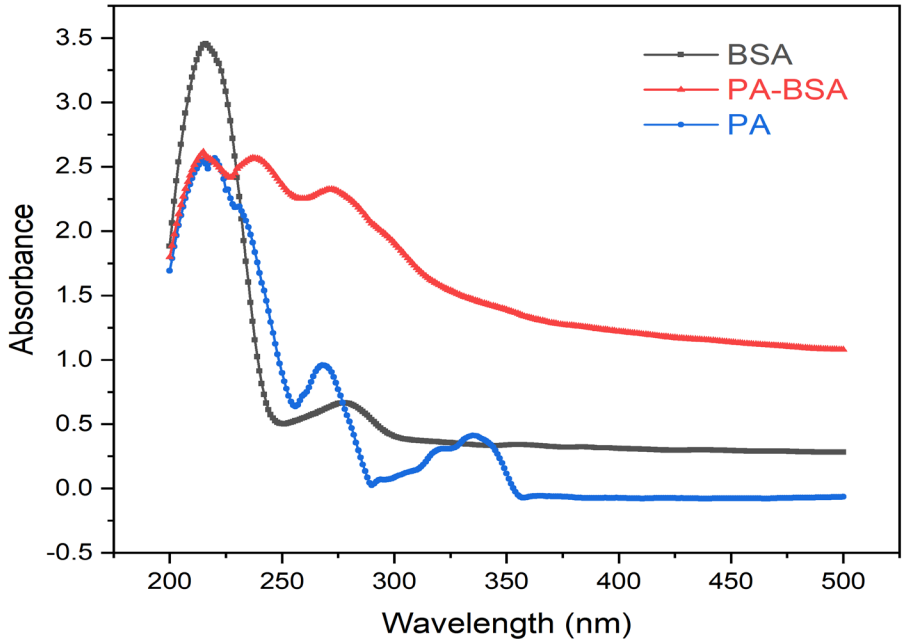
- **Immunogen: Bap-BSA**（Bovine Serum Albumin）



- **Coating antigen: PyBA-BSA**



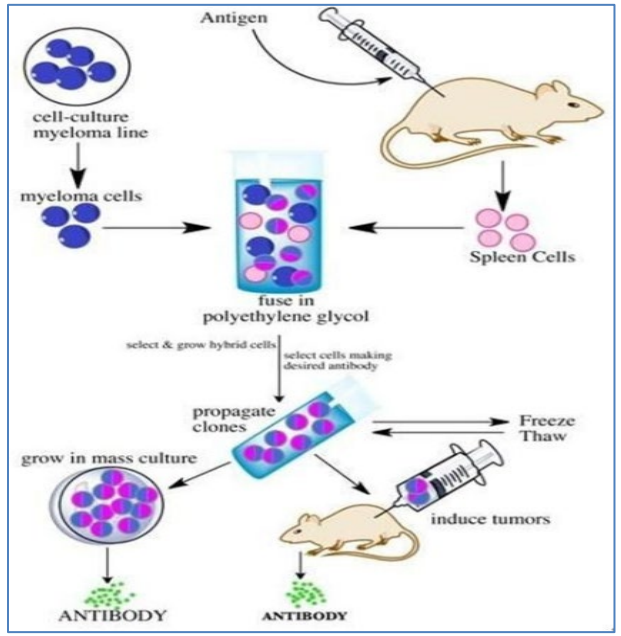
pyrene-1-butyric acid

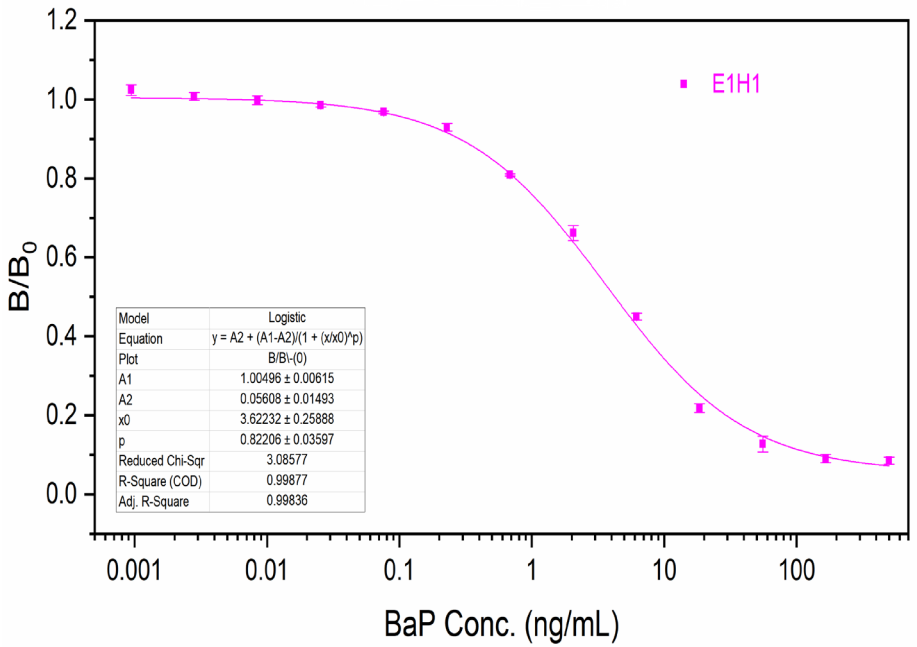


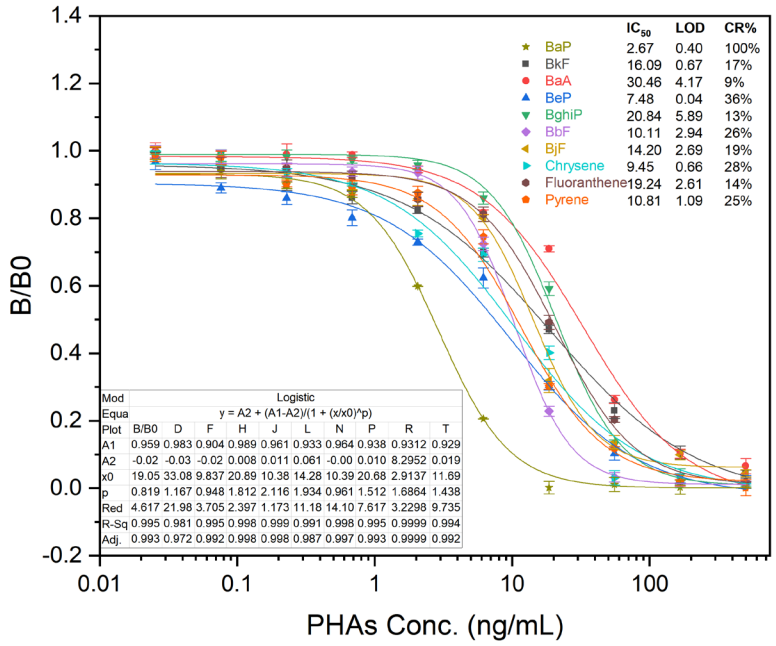
Preparation of anti-B[a]P mAbs

High quality monoclonal antibody of BaP named E1H11 was obtained

- **The half maximal inhibitory concentration(IC_{50})=2.67 ng/mL**
- **The CRs for anthracene, fluoranthracene, perylene, pyrene, etc. <15%**
- **Affinity constant is $1.2x10^5$**







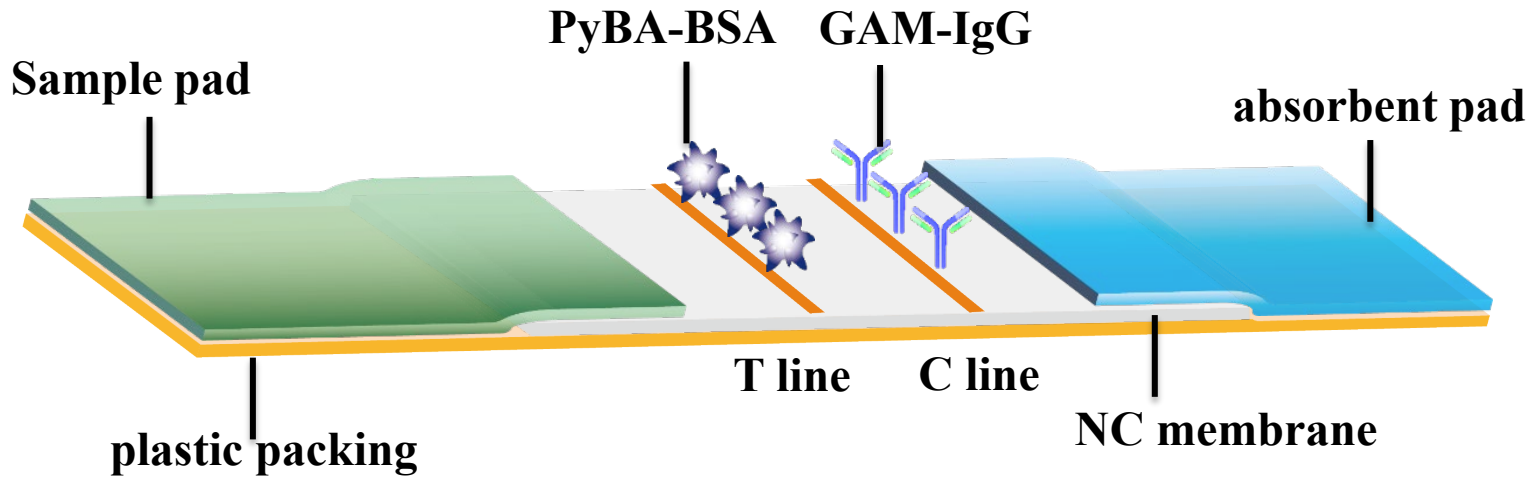
Establishment of time resolved fluorescence lateral flow immunoassay (TRFICA)

Assembly of the TRFICA strip

The strip consists of three components:

Plastic packing, NC membrane, and pads (sample and absorbent pads)

The control line (C line) and test line (T line) were sprayed with GAM-IgG and the coating antigen PyBA-BSA, respectively.

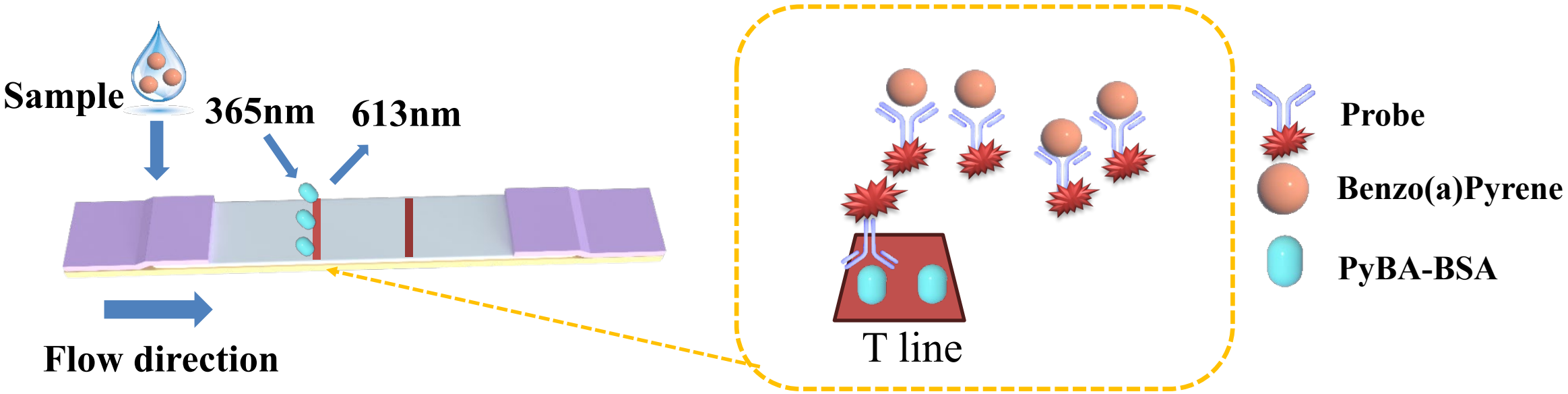


Principle of TRFICA detection for B(a)P

I. B(a)P, when present in a sample, and a PyBA-BSA compete for the binding sites of anti-B(a)P antibodies on T line, the antibodies are labeled with Eu/Tb(III) nanospheres served as probe, so a fluoresence signal is generated.

II. The intensity of the fluoresence signal is inversely proportional to the concentration of the B(a)P present in the sample.

III. The signal is evaluated using a portable strip reader. The concentrations of the Bap are determined by interpolation using the standard curve constructed with each run.

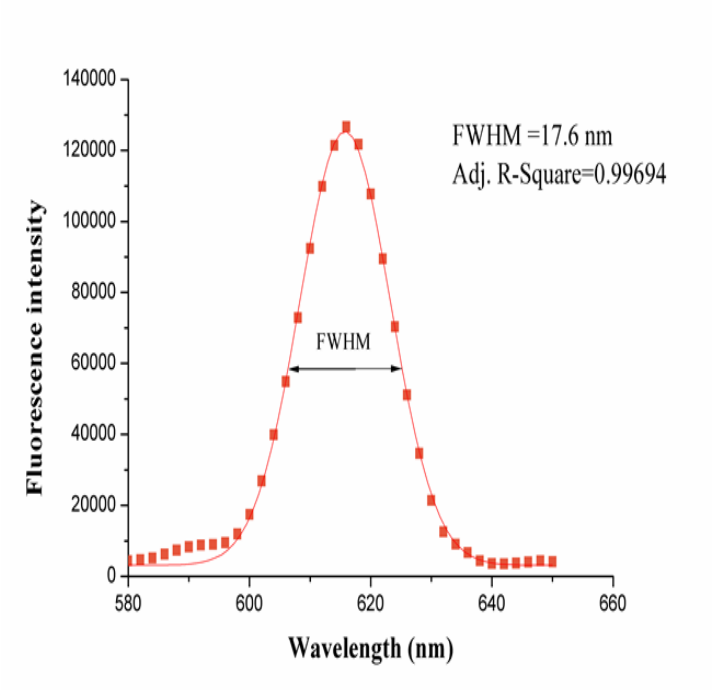


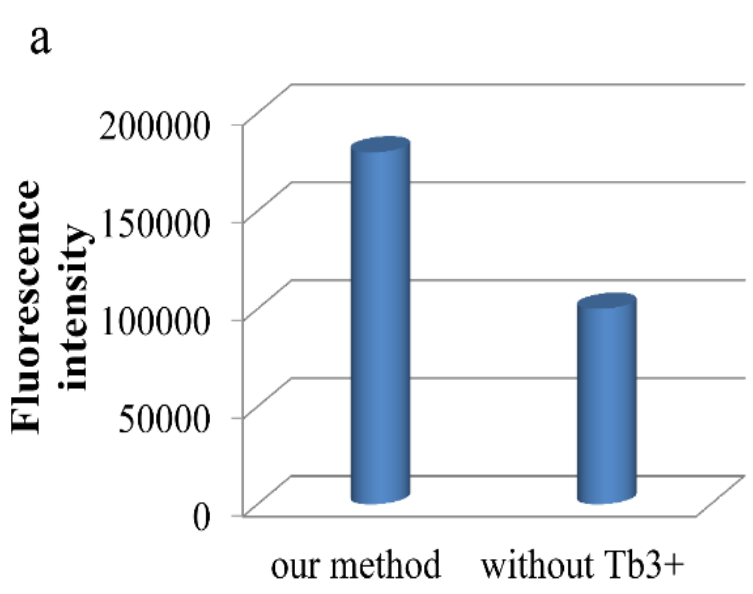
Preparation and characterization of Eu/Tb(III) nanospheres

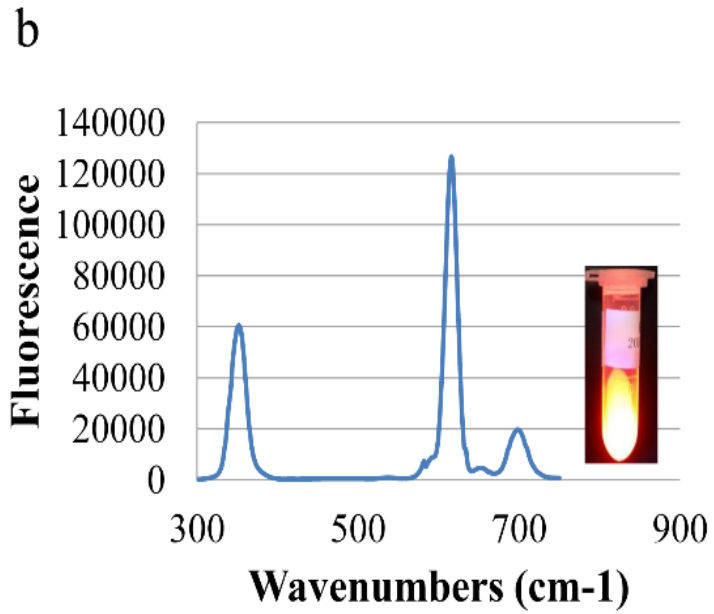
Optical properties of Eu/Tb(III) : Excited at 350 nm, emission at 618nm

Eu/Tb(III) with a satisfactory fluorescence intensity

Provides Fluorescent labeling materials for highly sensitive detection

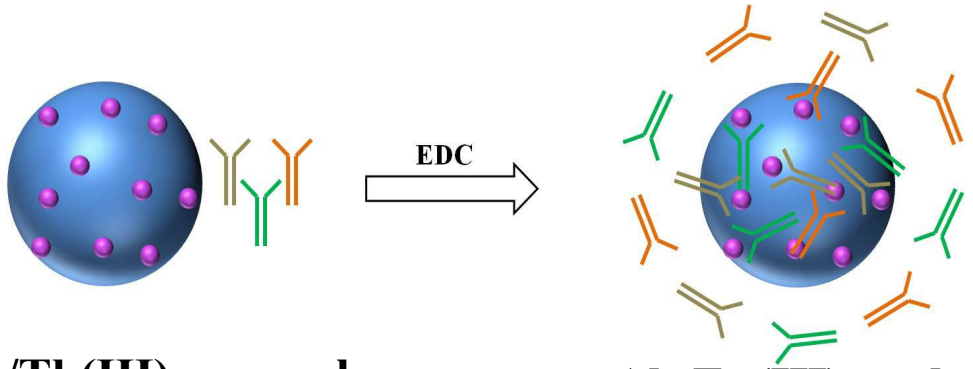




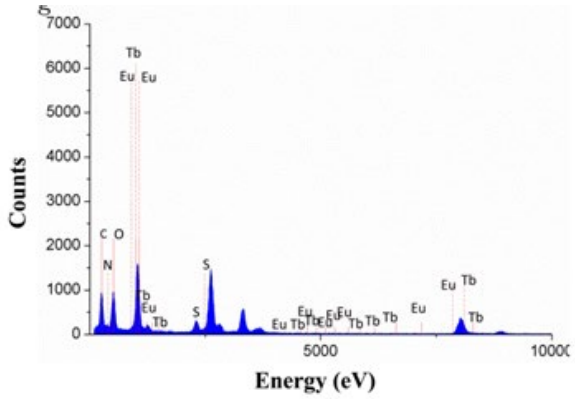


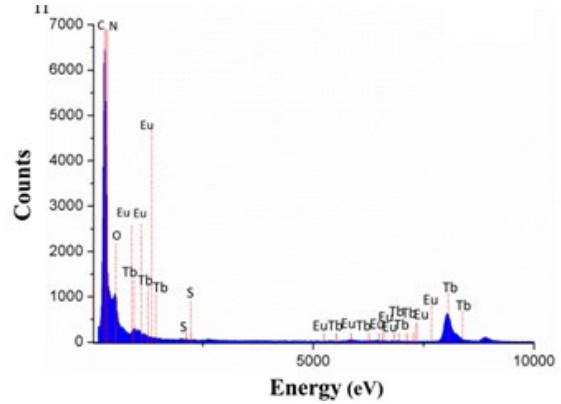
Identification of the Eu/Tb(III) -mAb probe

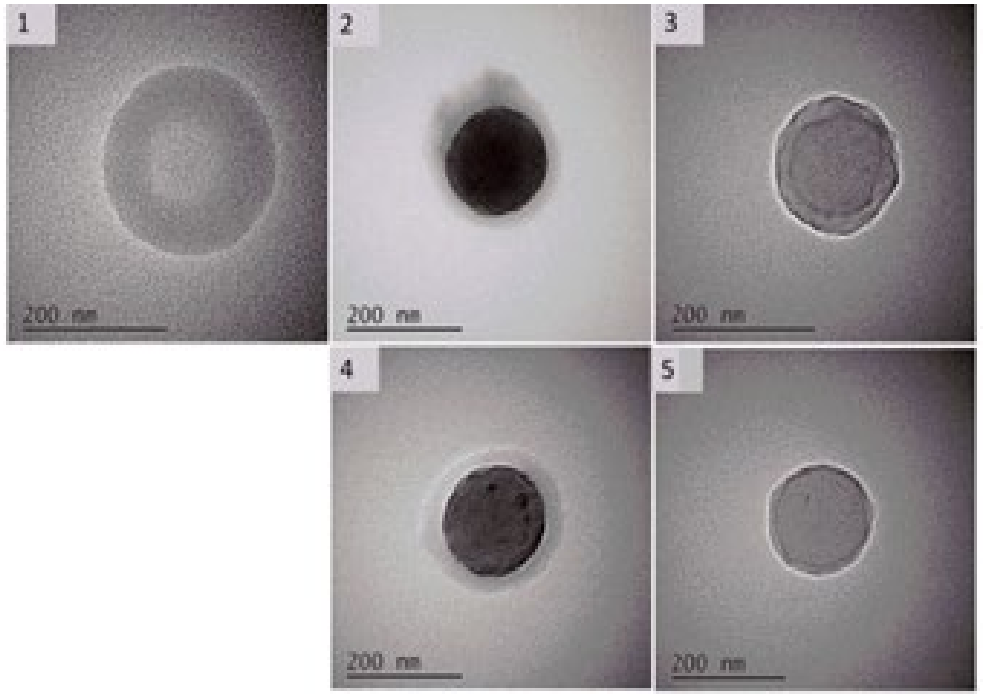
- The detection probe was generated by conjugating the Eu/Tb(III) and the mAb *via* EDC and NHS
- The TEM and EDS were performed to study the characterization of probes
- Characteristic peaks of C and N were obviously higher after conjugation



Eu/Tb(III) nanospheres **mAb-Eu(III) probe**







Optimization of TRFICA test strip

1. The concentration of immunoreagents

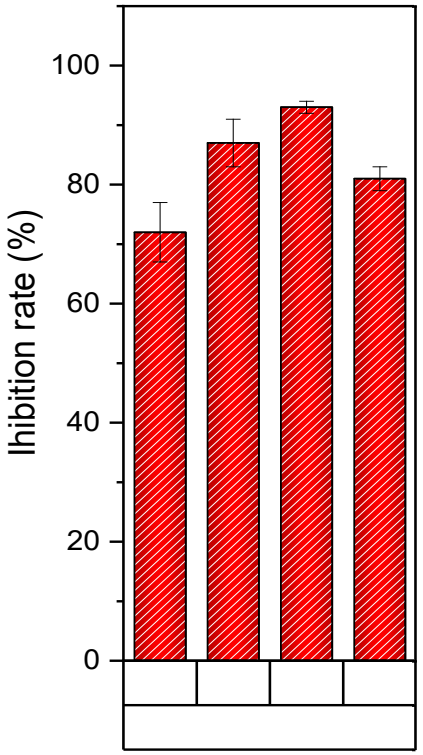
- T line: PyBA-BSA 0.8 mg/mL 0.7 μL/cm
- C line: GAM-IgG 0.005 mg/mL 0.6 μL/cm

BaP-BSA		immunoprobe dilution ratio	anti-IgG antibody	
conc. mg/mL	speed μL/cm		conc. mg/mL	speed μL/cm
0.8	0.7	200	0.005	0.6

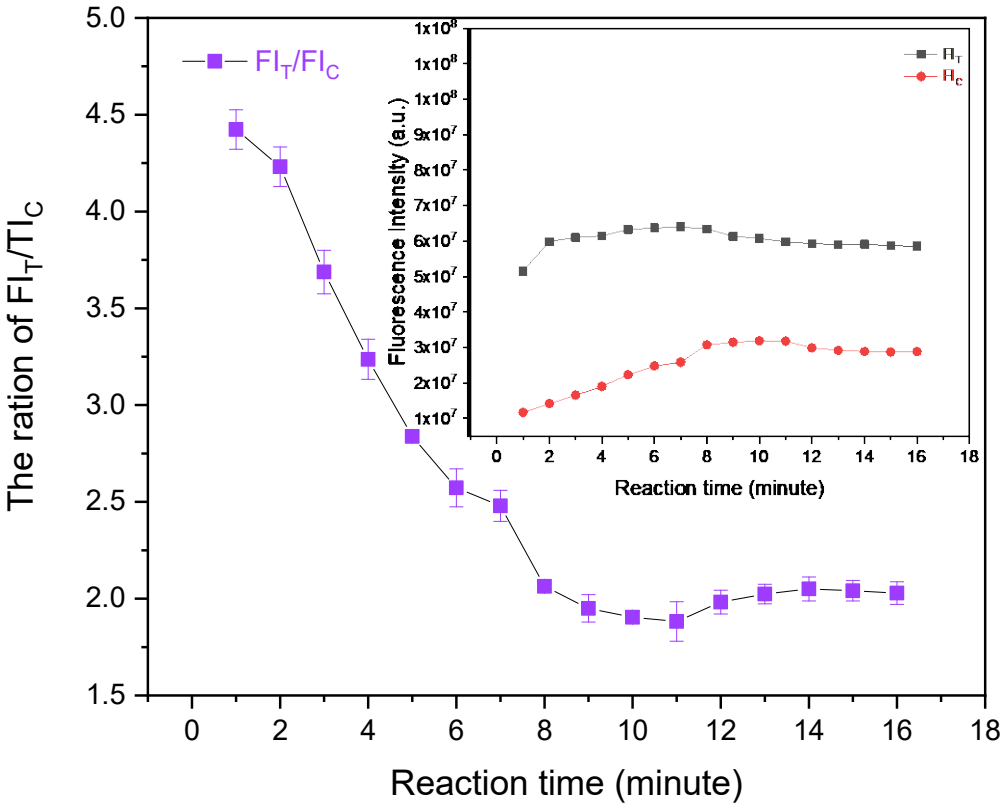
Optimization of TRFICA test strip

2. NC membrane and reaction time

HiFlow 120



Reaction time: 7 min



Optimization of TRFICA test strip

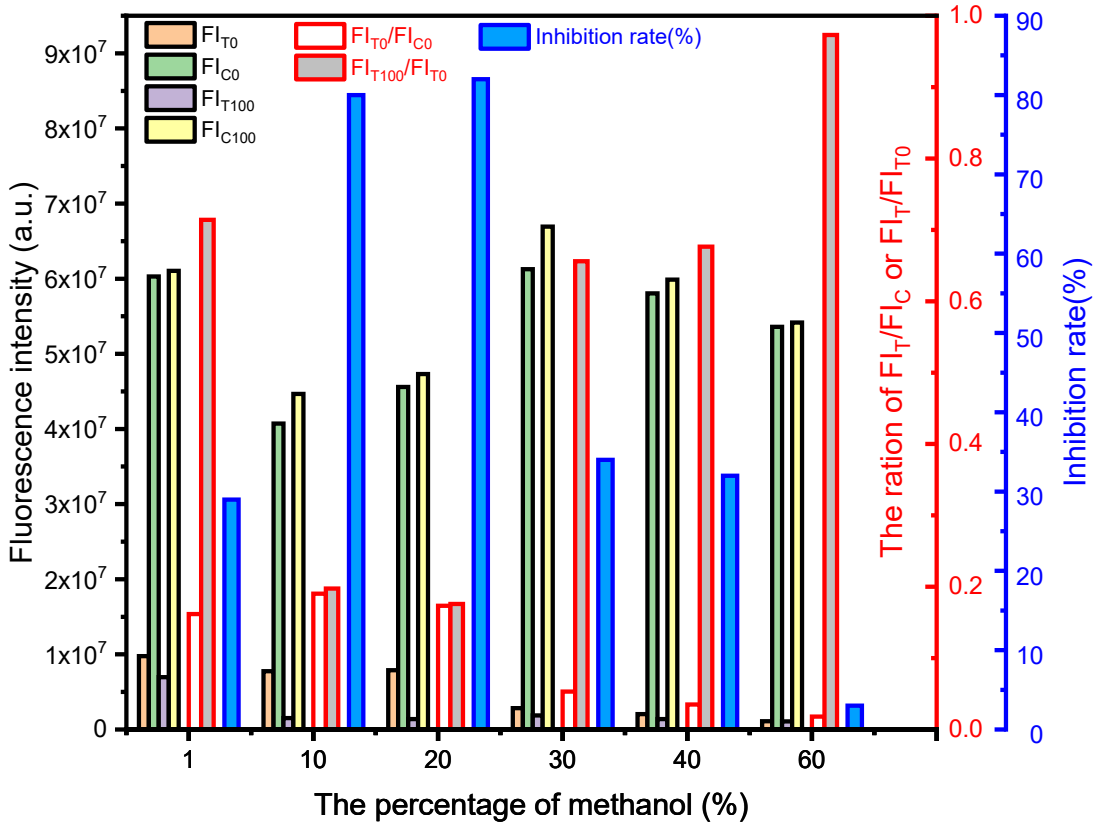
3. Detection solution composition

NO.	element	0 ppb		50 ppb		100 ppb		FI_{T100}/FI_{T0} (n=5)
		T	C	T	C	T	C	
1	1% sucrose+1% Tween-20+PBS	24150000	67700000	14290000	67660000	8060000	71510000	0.33
2	2% sucrose+1% Tween-20+PBS	9580000	63700000	8060000	60630000	2400000	60490000	0.25
3	1% sucrose+2.5% Tween-20+PBS	8930000	59860000	6240000	60350000	2130000	60400000	0.24
4	1% sucrose+2% Tween-20+ 1% PVPK-30+PBS	21880000	69240000	8160000	69600000	3070000	68410000	0.14
5	1% sucrose+2% Tween-20+ 1% PVPK-30+1% BSA+PBS	9060000	67700000	9060000	65610000	2100000	66490000	0.23
6	1% sucrose+2% Tween-20+ 1% PVPK-30+1% OVA+PBS	8840000	69710000	8020000	68100000	3020000	61410000	0.34

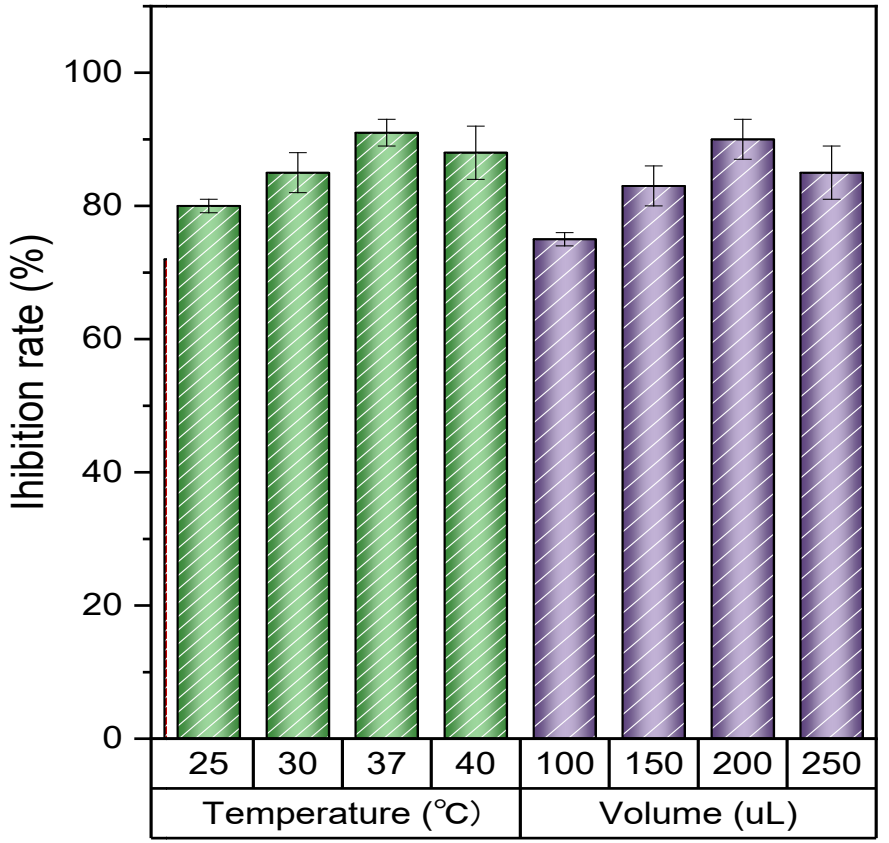
Optimization of TRFICA test strip

4. Solvent composition, incubation temperature and volume

Optimum solvent composition:
20% methanol

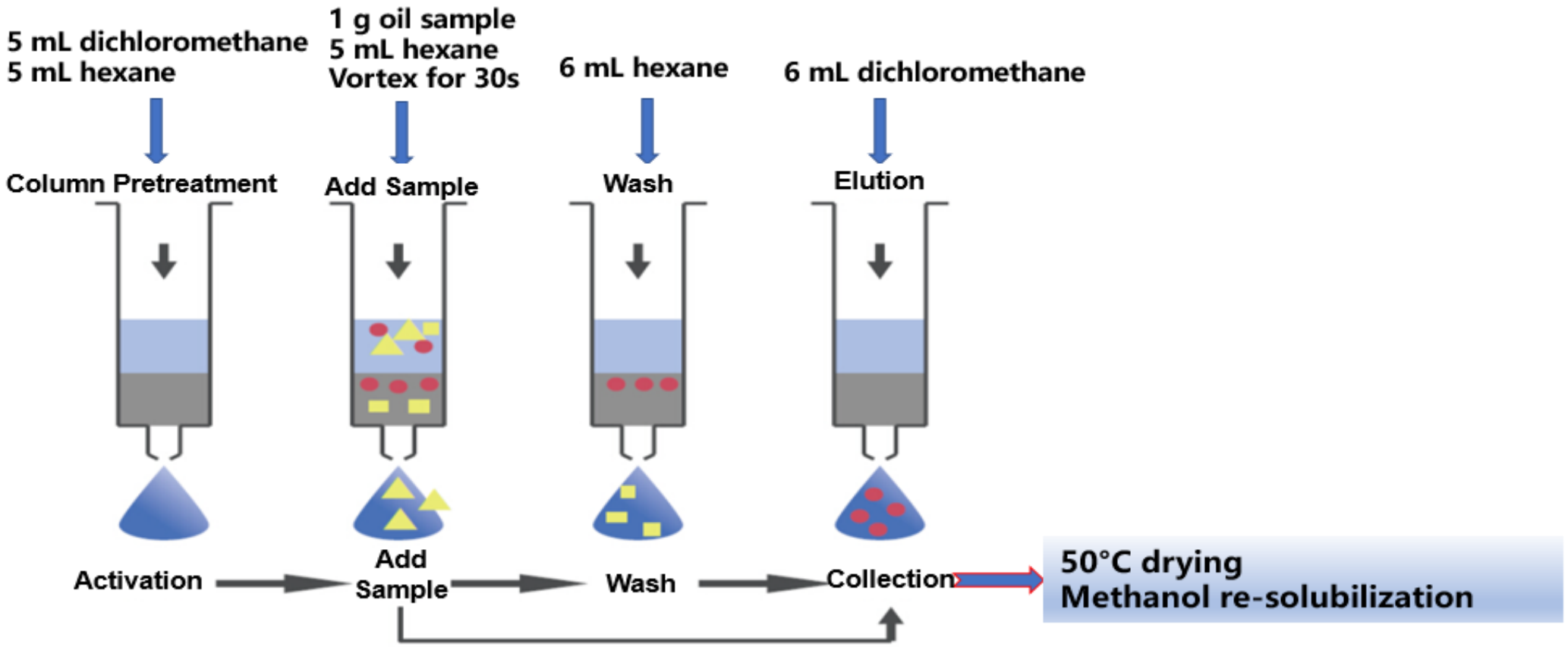


Incubation 37°C
Sample volume 200 μL



Sample preparation of TRFICA test strip

Molecularly imprinted purification (MIP) for BaP in edible oil



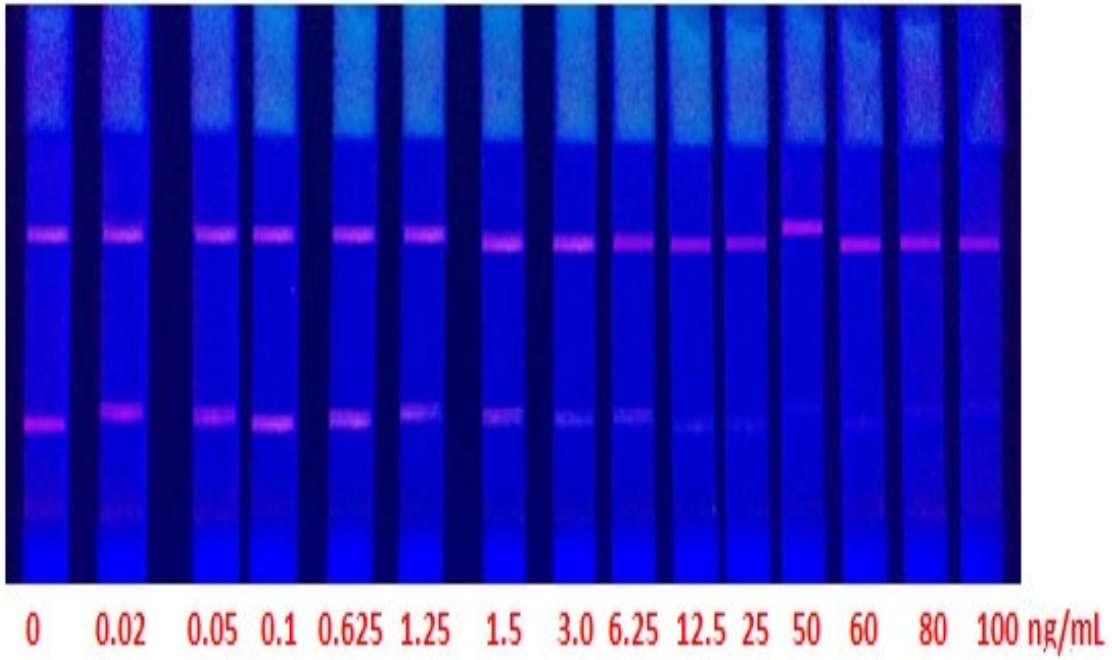
Evaluation of MIP pretreatment in different edible oils

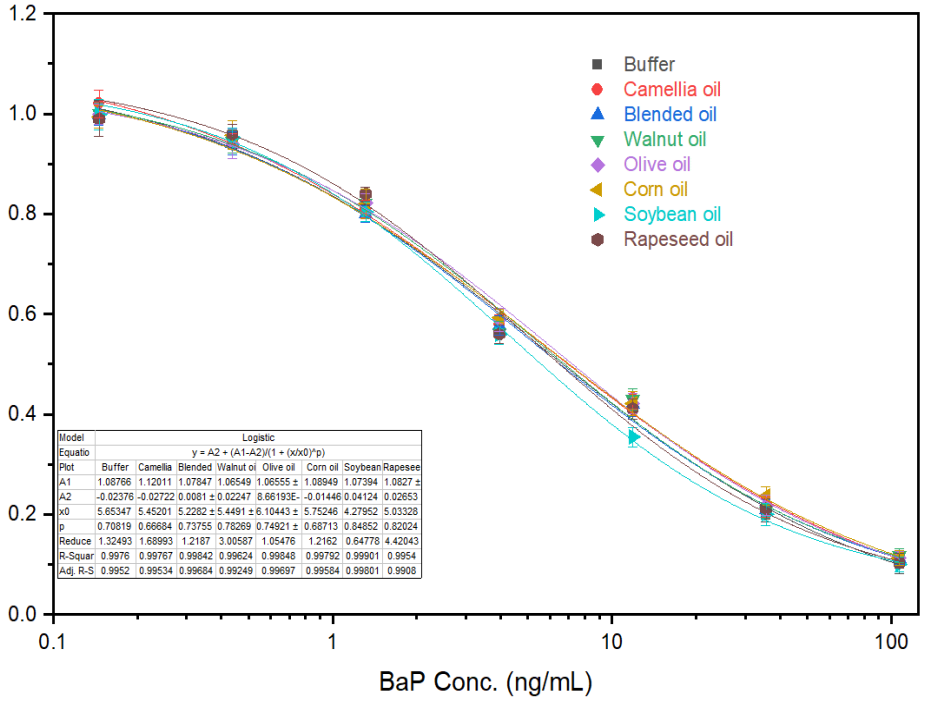
Recovery rate of rapeseed oil: 103-113.8%

Samples	Spiked (μg/kg)	HPLC found (μg/kg)	Recovery (%)	Spiked (μg/kg)	HPLC found (μg/kg)	Recovery (%)	Spiked (μg/kg)	HPLC found (μg/kg)	Recovery (%)
Soybean oil		3.34	111.21		4.26	85.2		10.26	102.6
Rapeseed oil		3.26	108.7		5.15	103.0		11.38	113.8
Corn oil	3.00	2.78	92.7	5.00	4.67	93.4	10.00	8.88	88.8
Peanut oil		3.19	106.4		4.29	85.7		10.18	101.8
Blended oil		2.73	91.0		5.03	100.5		11.51	115.1
Camellia oil		3.65	121.5		4.45	88.9		9.94	99.3
Walnut oil		3.15	105.1		5.75	114.9		9.85	98.5
Olive oil		3.18	106.1		4.39	87.7		11.14	111.4
Sunflower oil		2.83	94.2		5.09	101.7		10.26	102.6
Cottonseed oil		3.28	109.3		5.03	100.7		9.15	91.5

Establish calibration curve for B(a)P in rapeseed oil

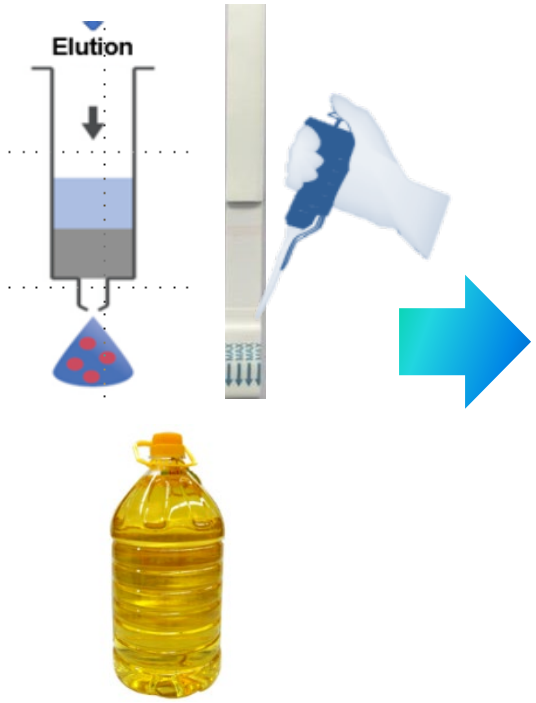
- Calibration curve were established by plotting the ratio between the intensity of T line and the C line (T/C) against the log of Bap concentration
- TRFICA for B(a)P : LOD 0.6 ng/mL, $R^2 = 0.9892$

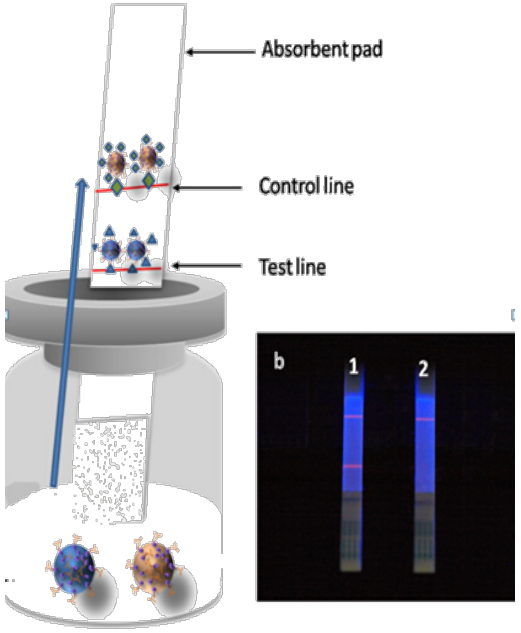




Model	Logistic							
Equatio	y = A2 + (A1-A2)/(1 + (x/x0)^p)							
Plot	Buffer	Camellia	Blended	Walnut oi	Olive oil	Corn oil	Soybean	Rapesee
A1	1.08766	1.12011	1.07847	1.06549	1.06555 ±	1.08949	1.07394	1.0827 ±
A2	-0.02376	-0.02722	0.0081 ±	0.02247	8.66193E-	-0.01446	0.04124	0.02653
x0	5.65347	5.45201	5.2282 ±	5.4491 ±	6.10443 ±	5.75246	4.27952	5.03328
p	0.70819	0.66684	0.73755	0.78269	0.74921 ±	0.68713	0.84852	0.82024
Reduce	1.32493	1.68993	1.2187	3.00587	1.05476	1.2162	0.64778	4.42043
R-Squar	0.9976	0.99767	0.99842	0.99624	0.99848	0.99792	0.99901	0.9954
Adj. R-S	0.9952	0.99534	0.99684	0.99249	0.99697	0.99584	0.99801	0.9908

TRFICA protocol for detection real sample





Sample preparation **Strip** **Reader** **Data**

Accuracy and precision of the TRFICA

- **The average recoveries of B[a]P were 86.1%–114.79% using TRFICA**
- **Relative coefficient of variation: 0.04–2.05%**

	spiked conc. (ng/0.5g)	theoratal level (ng/mL)	detection conc. (ng/mL)± SD	recovery (%)	CV (%)
intra-assay (n=5)	10	0.77	0.88±0.018	114.79	6.05
	50	19.23	17.20±0.032	89.45	8.19
	200	76.92	66.24±0.464	86.11	5.10
inter-assay (n=5)	10	3.85	3.71±0.025	96.36	7.67
	50	19.23	18.33±0.224	95.32	6.13
	200	76.92	69.71±0.532	90.63	7.04

B[a]P level in different kinds of rapeseed oil

- Hot press oil contained B[a]P approximately 2.18 μg kg^{-1}
- The cold drawn oil samples contained little B[a]P, exhibit less risk

Category of rapeseed oil	Sample No.	HPLC (ng/mL) ± SD	TRFICA (ng/mL)± SD
Hot press oil	1#	1.95±0.018	1.85±0.13
	2#	2.35±0.16	2.20±0.23
	3#	2.25±0.28	2.46±0.16
Cold drawn oil	1#	ND	ND
	2#	1.23±0.13	1.33±0.17
	3#	ND	ND

Conclusion

1. B[a]P hapten and the corresponding antigens were systhesized successfully
2. Sensitive and specific anti-B[a]P mAb was screened and purification
3. A rapid low-cost and easy method based on TRFICA for B[a]P in rapeseed oil was established with LOD of 0.6 ng/mL, reaction time only 7 min
4. Hot press oil contained B[a]P approximately 2.18 μg kg^{-1},while the cold drawn oil samples contained little B[a]P, exhibit less risk
5. The developed TRFICA is suitable for the sensitive detection of B[a]P in real-life rapeseed oil samples

Thank you for your attention


Wuhan China
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