INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SEAFOOD USING GC-MS: A COLLABORATIVE STUDY

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Talk Summary

- **AOAC CALL: PAHS IN FISH AND SEAFOOD**
- **NEW METHOD FOR MULTIPLE POPs**
- **AOAC: COLLABORATIVE STUDY**

Oil Spill - Gulf Of Mexico 2010

Call for Methods

23/7/2010

AOAC INTERNATIONAL

NEWS FLASH

Call for Methods

Methods for Measurement of Polycyclic Aromatic Hydrocarbon (PAH) Compounds in Gulf of Mexico Seafood

AOAC INTERNATIONAL is inviting method developers to submit methods for consideration and possible evaluation through the AOAC *Official Methods*SM program. Prospective methods must be able to quantify polycyclic aromatic hydrocarbon (PAH) compounds in the raw edible portions of fin fish, and in all of the tissues of crustaceans and shell fish (collectively "seafood"). Polycyclic aromatic hydrocarbon (PAH) compounds of interest are identified in Table 1. Acceptable methods must be able to demonstrate a Limit of Quantification[1] of 1 ppb (ng/g) for benzo(a)pyrene in seafood. Gulf of Mexico seafood species are of primary interest. Test samples are raw, homogenized seafood tissues. Currently accepted analytical methods require 96 to 120 hours to complete. Evaluation of analytical methods that significantly reduce the time-to-signal (including sample preparation and extraction) is a primary goal of this call for methods.

Table 1: Polycyclic Aromatic Hydrocarbon (PAH) Compounds of Concern

- Anthracene/phenanthrene
- Benz(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(g,h,i)perylene
- Chrysene
- Dibenz(a,h)anthracene
- Fluorene
- Flouranthene
- Ideno(1,2,3-cd)pyrene
- Naphthalene
- Pyrene

CONffIDENCE

Integrated sample preparation

BFR | PCB | PAH | Non-*ortho* PCB

Extraction
Shaking (H_2O + ethylacetate)

Isolation
10 min

Partition (transfer into organic phase)
induced by $MgSO_4$ + NaCl

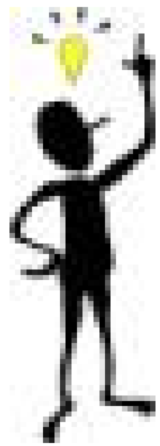
Clean up
Silicagel minicolumn

Clean up
30 min

Identification & quantification
GC-QMS(EI)
GC-TOFMS (EI)
GC×GC-TOFMS (EI)

Identification & quantification
1 h

6 SAMPLES / < 1 HOUR



SINGLE GC INJECTION

ICT PRAGUE

Target analytes

15 + 1 EU PAHs

Benz[a]anthracene	BaA
Benzo[b]fluoranthene	BbFA
Benzo[k]fluoranthene	BkFA
Benzo[ghi]perylene	BghiP
Benzo[a]pyrene	BaP
Chrysene	CHR
Dibenz[a,h]anthracene	DBahA
Indeno[1,2,3-cd]pyrene	IP
Benzo[j]fluoranthene	BjFA
Benzo[c]fluorene	BcFL
Cyclopenta[cd]pyrene	CPP
Dibenzo[a,e]pyrene	DBaeP
Dibenzo[a,h]pyrene	DBahP
Dibenzo[a,i]pyrene	DBaiP
Dibenzo[a,l]pyrene	DBalP
5-Methylchrysene	5-MC

Benzo[e]pyrene	BeP
Dibenzothiophene	DBT

16 US EPA PAHs

Acenaphthene	AC
Acenaphthylene	ACL
Anthracene	AN
Fluoranthene	FA
Fluorene	FL
Naphthalene	NA
Phenanthrene	PHE
Pyrene	PY

methylated homologues

2-Methylanthracene	2-MA
1-Methylchrysene	1-MC
3-Methylchrysene	3-MC
1-Methylnaphthalene	1-MN
2-Methylnaphthalene	2-MN
1-Methylphenanthrene	1-MPH
1-Methylpyrene	1-MP
1,7-Dimethylphenanthracene	1,7-DMP
2,6-Dimethylnaphthalene	2,6-DMN

Method Performance Characteristics – PAHs and their homologues (GC-TOFMS)

SHRIMPS

Recovery: 69 – 109 %

Repeatability: 2 – 15 %

LOQ: 0.05 – 0.25 μg.kg^{-1}

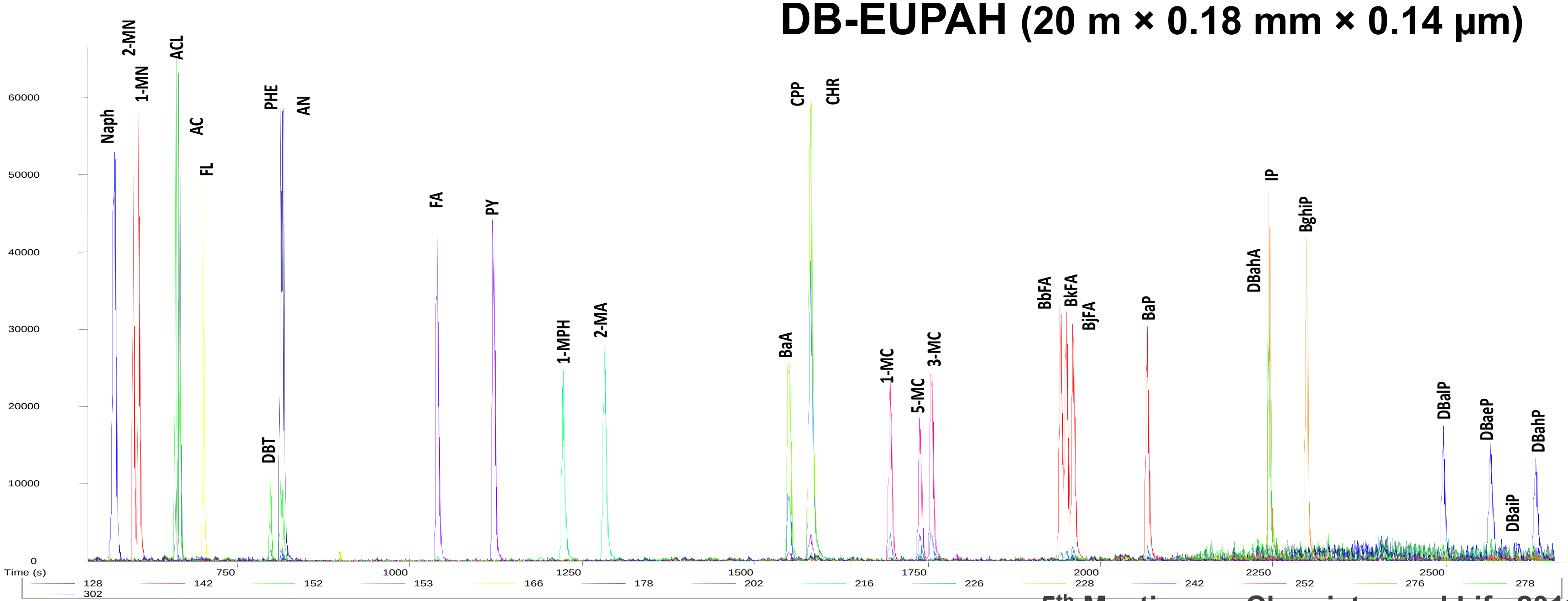
FISH

Recovery: 75 – 107 %

Repeatability: 2 – 13 %

LOQ: 0.05 – 0.25 μg.kg^{-1}

DB-EUPAH (20 m × 0.18 mm × 0.14 μm)



AOAC INTERNATIONAL

NEWS FLASH

21/10/2010

PAH Update: Candidate Method to Enter Collaborative Study

Due to the urgent need for rugged, reliable methods to determine polycyclic aromatic hydrocarbon (PAH) compounds in seafood from the Gulf, AOAC expedited a process that, ultimately, led to a candidate method ready for AOAC validation. AOAC facilitated a stakeholder panel and working group meetings; established a fitness-for-purpose statement; issued calls for methods and collaborators; evaluated available methodology purported to meet fitness for purpose; and selected the best candidate method for further evaluation and validation—all within 3 months. Further, AOAC has developed, and is currently finalizing, a validation study protocol, and the method is about to enter into collaborative study. AOAC validation of a method to detect PAHs in seafood is expected to take less than 6 months from start to finish.

In choosing a candidate method, AOAC reviewed approximately 30 methods for the detection of PAHs. Consequently, the PAH Working Group on Quantitative Methods, chaired by **Gina Ylitalo**, NOAA NWSFC, recommended a method by **Lucie Drabova et al.** at the Institute of Chemical Technology in Prague, Czech Republic as the most promising candidate method for further evaluation and, ultimately, validation as an AOAC-approved method.

In general, the method (Rapid Method for Simultaneous Determination of PAHs, Polychlorinated Biphenyls, and Polybrominated Diphenyl Ethers in Fish and Seafood Using GC-TOF/MS) is easy to perform, uses common laboratory equipment, and meets fitness-for-purpose and AOAC single-laboratory validation (SLV) requirements. The method uses a gas chromatography system coupled to a mass spectrometer detector that allows identification and quantification of all target PAHs.

AOAC INTERNATIONAL Collaborative Study

Final Protocol

Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood using Gas Chromatography-Mass Spectrometry: A Collaborative Study

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Introduction

Within a European integrated project CONffIDENCE (Contaminants in food and feed: Inexpensive detection for control of exposure), Jana Hajslova's group at the Institute of Chemical Technology (ICT) in Prague, Czech Republic developed a method for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood using gas chromatography coupled with time-of-flight mass spectrometry (GC-TOFMS). This method was selected for further study as an AOAC collaborative study by the AOAC Stakeholders Panel on Seafood Contaminants (SPSC), which was formed as a response to the seafood contamination resulting from the recent oil spill in the Gulf of Mexico. The analytes for this collaborative study have been narrowed down to include only PAHs and some of the relevant PAH alkyl homologues. Having a rapid method is essential for quick determination of contaminants in food, especially after environmental disasters. The nineteen contaminants found in Table 1 will be studied in this collaborative study.

Target analytes – AOAC study

1,7-Dimethylphenanthracene	1,7-DMP
1-Methylnaphthalene	1-MN
1-Methylphenanthrene	1-MPH
2,6-Dimethylnaphthalene	2,6-DMN
3-Methylchrysene	3-MC
Anthracene	AN
Benz[a]anthracene	BaA
Benzo[a]pyrene	BaP
Benzo[b]fluoranthene	BbFA
Benzo[ghi]perylene	BghiP
Benzo[k]fluoranthene	BkFA
Chrysene	CHR
Dibenz[a,h]anthracene	DBahA
Fluoranthene	FA
Fluorene	FL
Indeno[1,2,3-cd]pyrene	IP
Naphthalene	NA
Phenanthrene	PHE
Pyrene	PY

19 PAHs

AOAC Int. Collaborative Study

Study phases:

(1) Laboratory qualification

(2) Test sample analysis

Study design:

- 3 matrices: mussel, oyster, shrimp
- total of 5 different levels of BaP (2 – 50 µg/kg)
- other studied PAHs at varying levels from 2 to 250 µg/kg that mimic typical PAH patterns
- each matrix fortified at 3 different concentration levels in duplicate + one blank for each matrix
- total of 7 x 3 = 21 study samples

AOAC Int. Collaborative Study

Study participants:

- Adpen Laboratories (FL, USA)
- Covance Laboratories (WI, USA)
- EU PAH Reference Laboratory (Belgium)
- Eurofins CAL (LA, USA)
- FL Dept. of Agriculture and Consumer Services (FL, USA)
- Food Safety Net Services (TX, USA)
- **Institute of Chemical Technology (Czech Republic)**
- LECO Corporation (MI, USA)
- MD Dept. of Agriculture (MD, USA)
- MI Dept. of Community Health (MI, USA)
- Microbac Laboratories (IN, USA)
- Premier Laboratory (CT, USA)
- Silliker JR Laboratories (BC, Canada)
- Thermo Fisher Scientific FSRC (Germany)
- State Veterinary Institute in Praha, (Czech Republic)

15 laboratories

Study direction team:

- Co-study directors: K. Mastovska (Covance), W. Sorenson (Covance), and J. Hajslova (ICT)
- Technical advisors: J. Schmitz (Covance), J. Pulkrabova (ICT)

(1) Laboratory qualification phase

- Optimization of GC-MS, silica-SPE clean-up and solvent evaporation conditions
- Check of potential reagent blank contamination
- Familiarization with the method

- **Qualification steps:**

(1) GC separation test
(2) Calibration range test
(3) Solvent evaporation test
(4) PAH and fat elution profiles
(5) Procedure blank test
(6) Low-level spike test
(7) Practice sample analysis

1. GC Separation Test

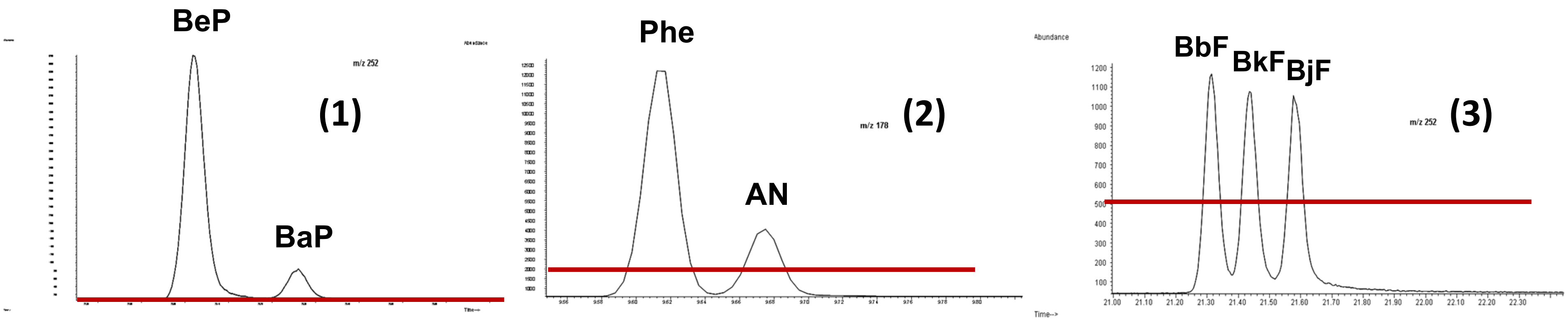
To optimize GC separation of PAHs

Criteria:

(1) a **baseline** separation of **benzo(a)pyrene** and **benzo(e)pyrene**

(2) at least **50%** valley separation of **anthracene** and **phenanthrene**

(3) at least **50%** valley separation for **benzo(b)fluoranthene, benzo(j)fluoranthene,** and **benzo(k)fluoranthene**



GC columns used in the study

(1) DB-EUPAH (20m x 0.18 mm x 0.14 µm) (Agilent J&W, USA)

(2) Rxi-17 Sil (30m x 0.25 mm x 0.25 µm) (Restek, USA)

(3) DB-17 MS (30m x 0.25 mm x 0.25 µm) (Agilent J&W, USA)

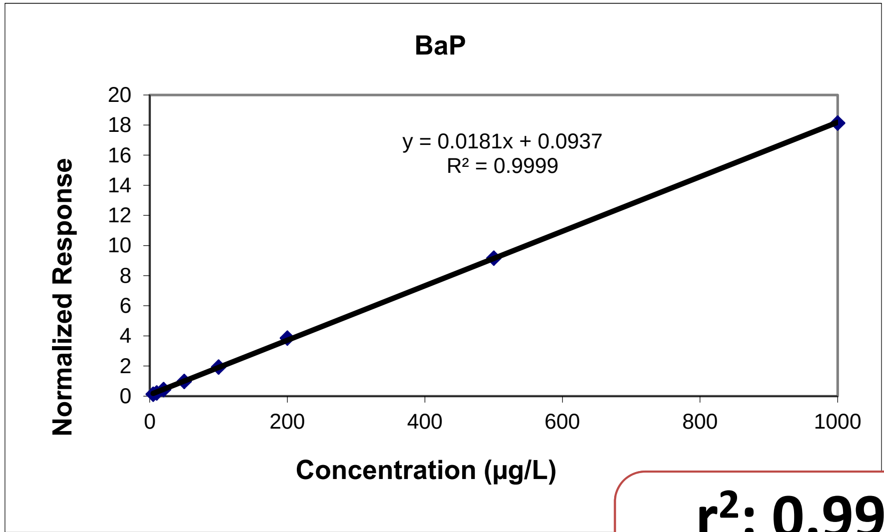
(4) TR-50MS (30m x 0.25 mm x 0.25 µm) (Thermo Fisher Scientific, USA)

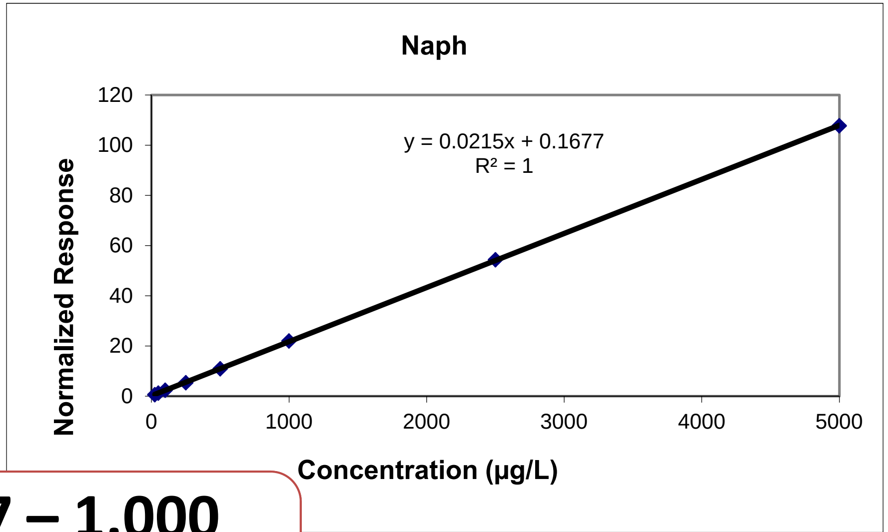
(5) ZB-50 (30m x 0.25 mm x 0.25 µm) (Phenomenex, USA)

2. Calibration Range Test

To determine linear range for analyte responses normalized to respective ^{13}C-PAHs, test carry-over, and optimize injection conditions

- **8-point calibration curves**
- **Calibration range:** 5 –1000 µg/L for BaP and lower levels PAH, 12.5 – 2500 µg/L for higher-level PAHs, 25 –5000 µg/L for naphthalene
- **Coefficients of determination (r^2)** of 0.990 or greater are required
- **Test for carry-over** (response in the solvent blank < 0.5% of the highest standard)





r^2: 0.9987 – 1.000

Max residuals ± 20%

3. Solvent Evaporation Test

To determine absolute recoveries of PAHs and ^{13}C-PAHs during two evaporation experiments simulating the two evaporation steps in the method

(a) evaporation of 5 mL of an PAH/^{13}C-PAH solution in EtOAc and reconstitution in isooctane

(b) evaporation of 10 mL of an PAH/^{13}C-PAH solution in hexane:DCM (3:1, *v/v*) and reconstitution in isooctane

Criteria:

- The absolute recoveries - above 70%
- Recoveries of the heavier PAHs - in the range of 90-110%

Evaporation techniques employed in the study:

- nitrogen blown-down
- rotary vacuum evaporation

Recoveries : 78 – 109 %

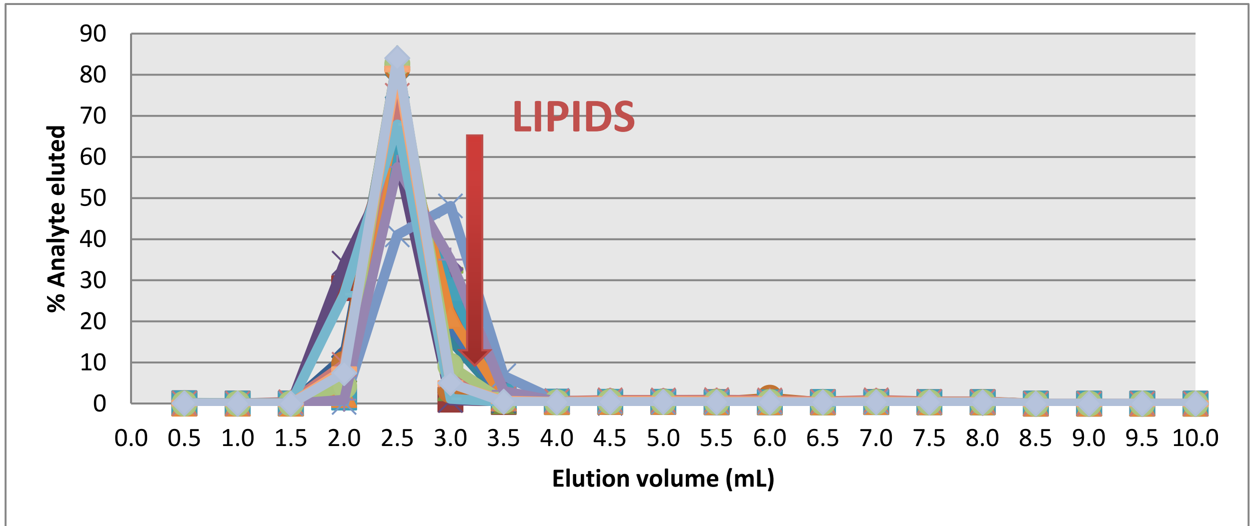
Recommendations:

- use isooctane as a keeper in both evaporation steps
- add 1-2 mL of EtOAc prior to the second evaporation step to improve recoveries of volatile PAHs

4. PAH and Fat Elution Profiles

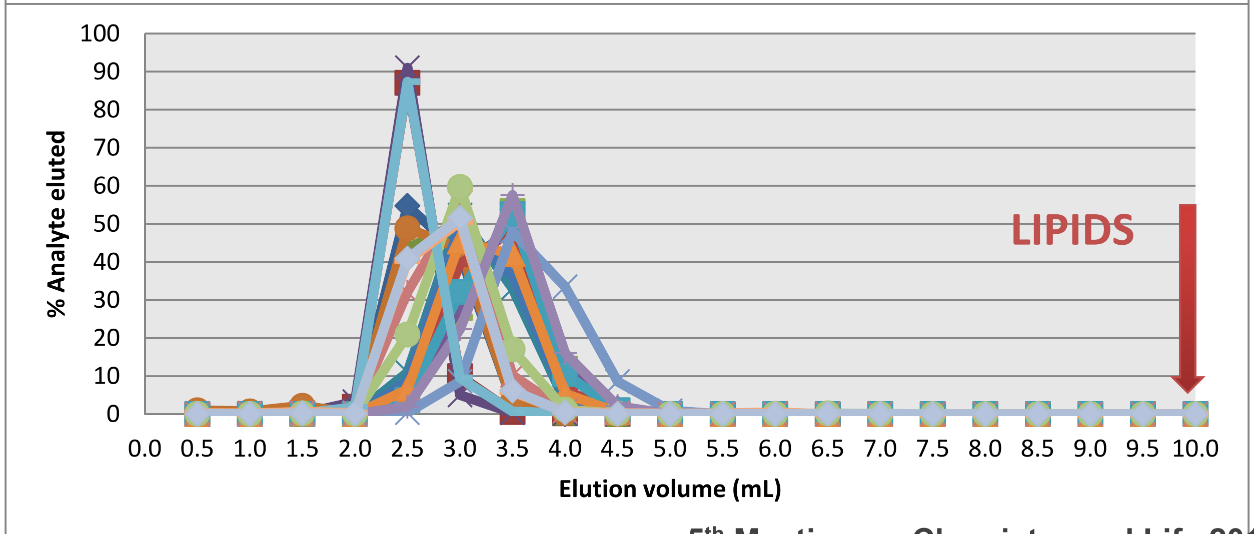
To determine elution profiles of PAHs and fat on the silica gel SPE cartridge of your choice and optimize the elution solvent volume

Commercial SPE silica cartridge (UCT)



100 mg of fat loaded on column containing 1G of sorbent

in-house prepared mini - column



5. Procedure Blank Test

To evaluate and minimize background contamination, potentially occurring from different sources

Criteria:

- Concentration of PAHs in blank < lowest calibration level
- Concentration of Naph < 50 µg/mL (equivalent to 5 µg/g sample)

Potential contamination sources:

- Laboratory air
- Solvents
- Salts (have to be muffled)
- Glassware
- Extraction tubes (certain PAHs released from contaminated tubes when heated by the exothermic reaction caused by addition of anhydrous $MgSO_4$ to the aqueous extract)

6. Low-Level Spike Test

Analyze 7 replicates of a blank shrimp matrix spiked at PAH concentrations equivalent to the fitness-for-purpose LOQ for BaP of 1 µg/kg and corresponding concentrations of other PAHs (based on their concentration ratio in the calibration solutions)

	Average recovery (%, n = 7)								
PAH	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 7**	**Mean**	**RSD (%)**
1-MN	167	127	124	106	110	83	95	**116**	23
Ant	105	98	94	84	93	96	91	**94**	6
BaP	103	85	89	82	92	98	85	**90**	8
BghiP	109	111	88	80	92	106	82	**95**	14

Recoveries : 90 – 139 %

Repeatability: 8 – 23 %

7. Practice Samples

Familiarization with the complete analysis procedure. This will ensure that instrumentation and reagents are all working properly in advance of conducting the actual study

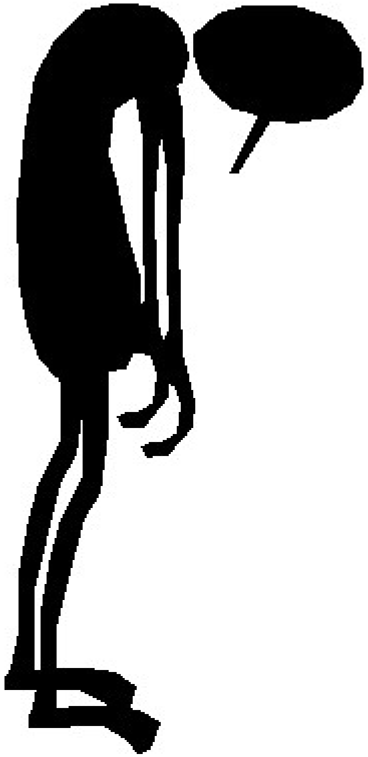
- Three samples of two varying matrices
- *Sample of NIST SRM 1974b - Organics in Mussel Tissue (Mytilus edulis)*

		NIST SRM 1974b - Recoveries (%)									
PAH	**µg/kg**	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 7**	**Lab 8**	**Mean**	**RSD (%)**
BaA	4.74	90	83	80	86	73	93	85	89	**85**	7
BaP	2.8	99	80	79	76	72	79	76	89	**81**	11
BghiP	3.12	103	99	93	96	84	100	101	109	**98**	8
Flt	17.1	104	102	93	102	85	100	103	103	**99**	7

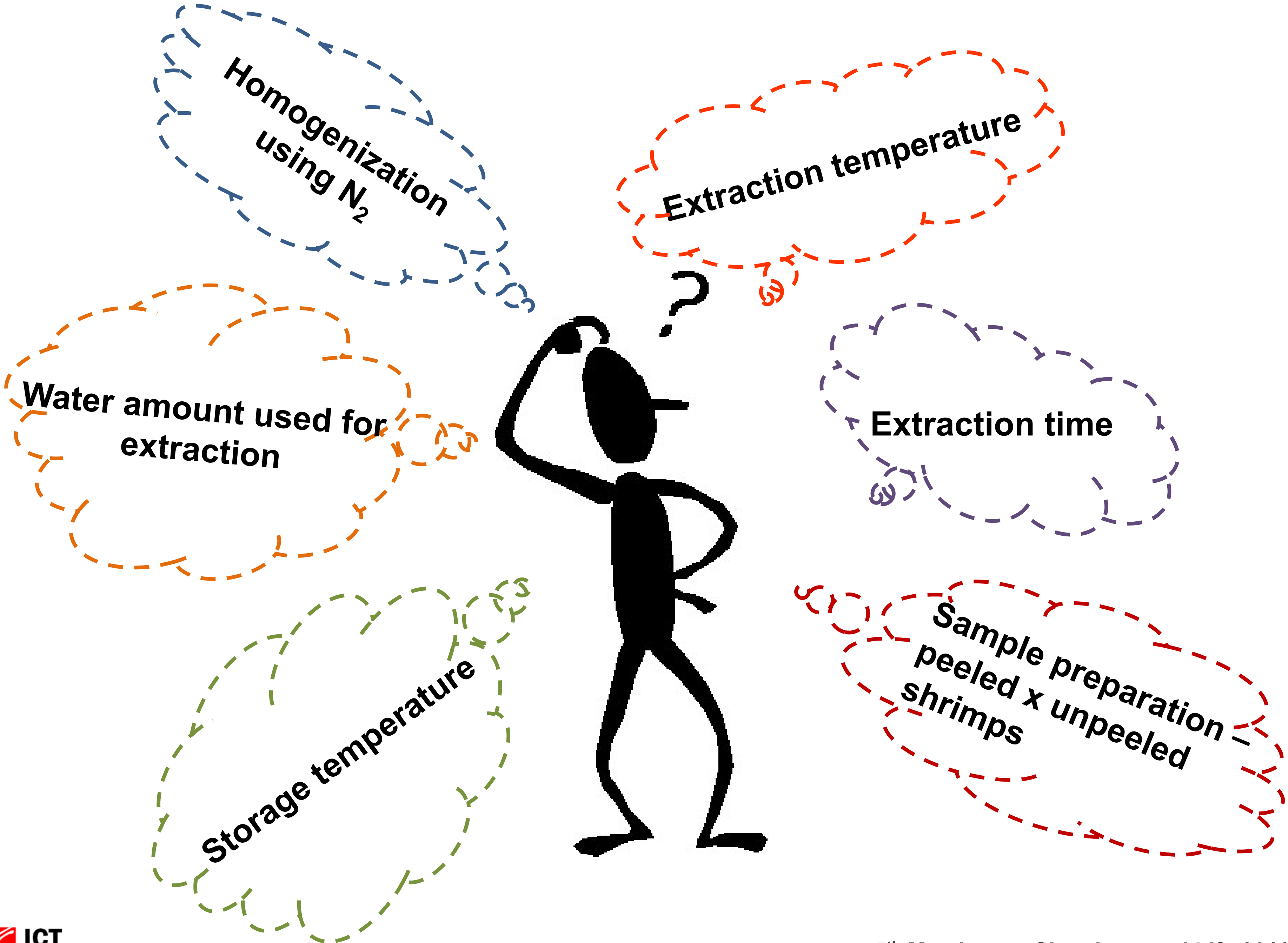
- *Two samples of spiked shrimps*

	SHRIMP Practice Sample - Recoveries (%)								
PAH	**Lab 1**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 7**	**Mean**	**RSD (%)**
1-MN	81	82	88	122	77	57	146	**93**	33
Ant	63	71	75	89	66	58	59	**69**	16
BaP	61	71	63	74	57	38	51	**59**	20
BghiP	60	74	62	72	57	39	50	**59**	21

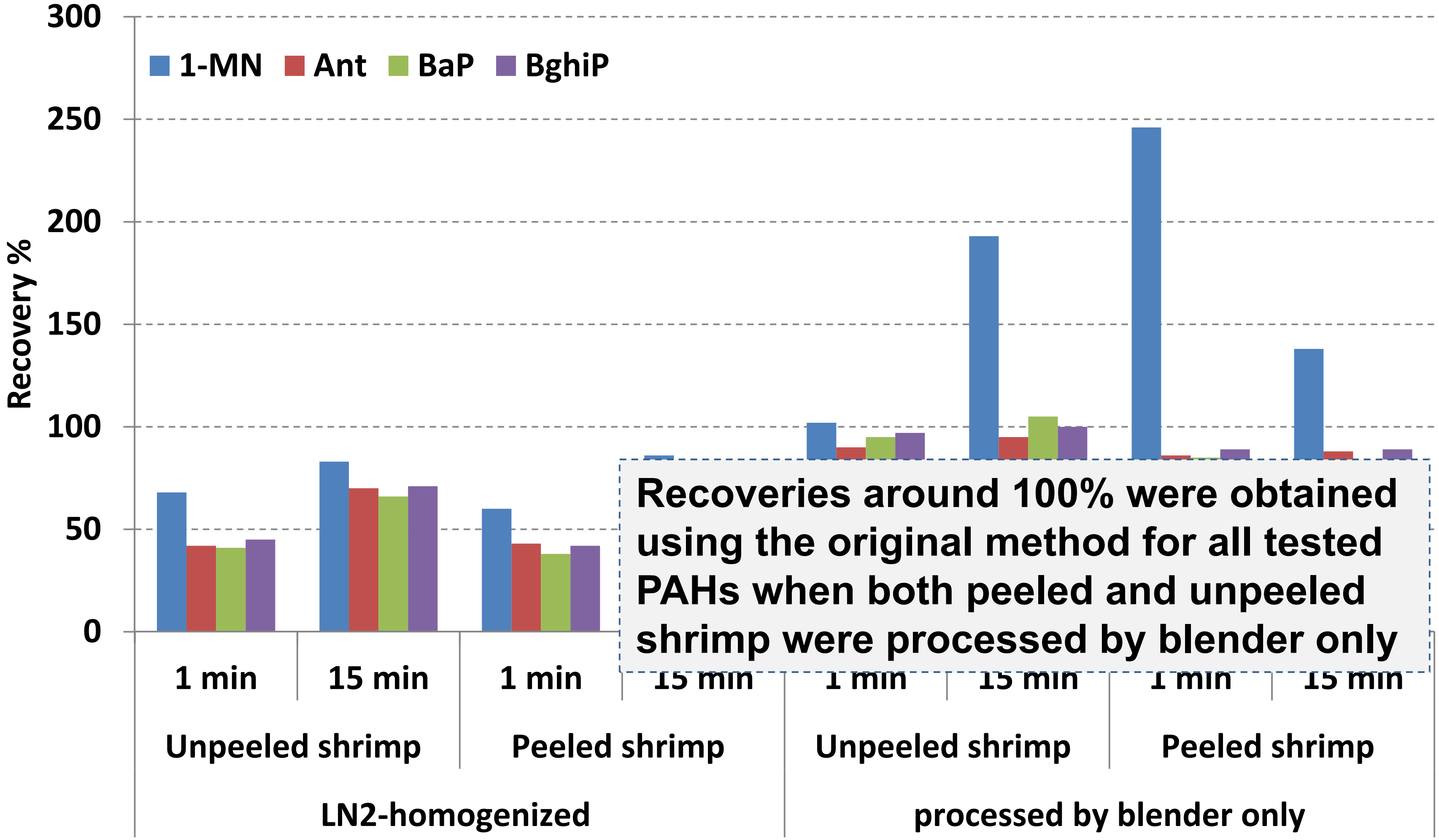
Low-Level Spike Test and Practice samples



	Recovery %	
PAH	low level spikes	practice samples-spiked shrimps
1,7-DMP	84	48
1-MN	103	93
1-MP	92	62
2,6-DMN	96	48
3-MC	81	61
Ant	105	69
BaA	101	56
BaP	**103**	**59**
BbF	104	56
BghiP	109	59
BkF	101	58
Chr	105	56
DBahA	102	57
Fln	91	68
Flt	107	58
IcdP	102	57
Naph	113	85
Phe	92	65
Pyr	107	59

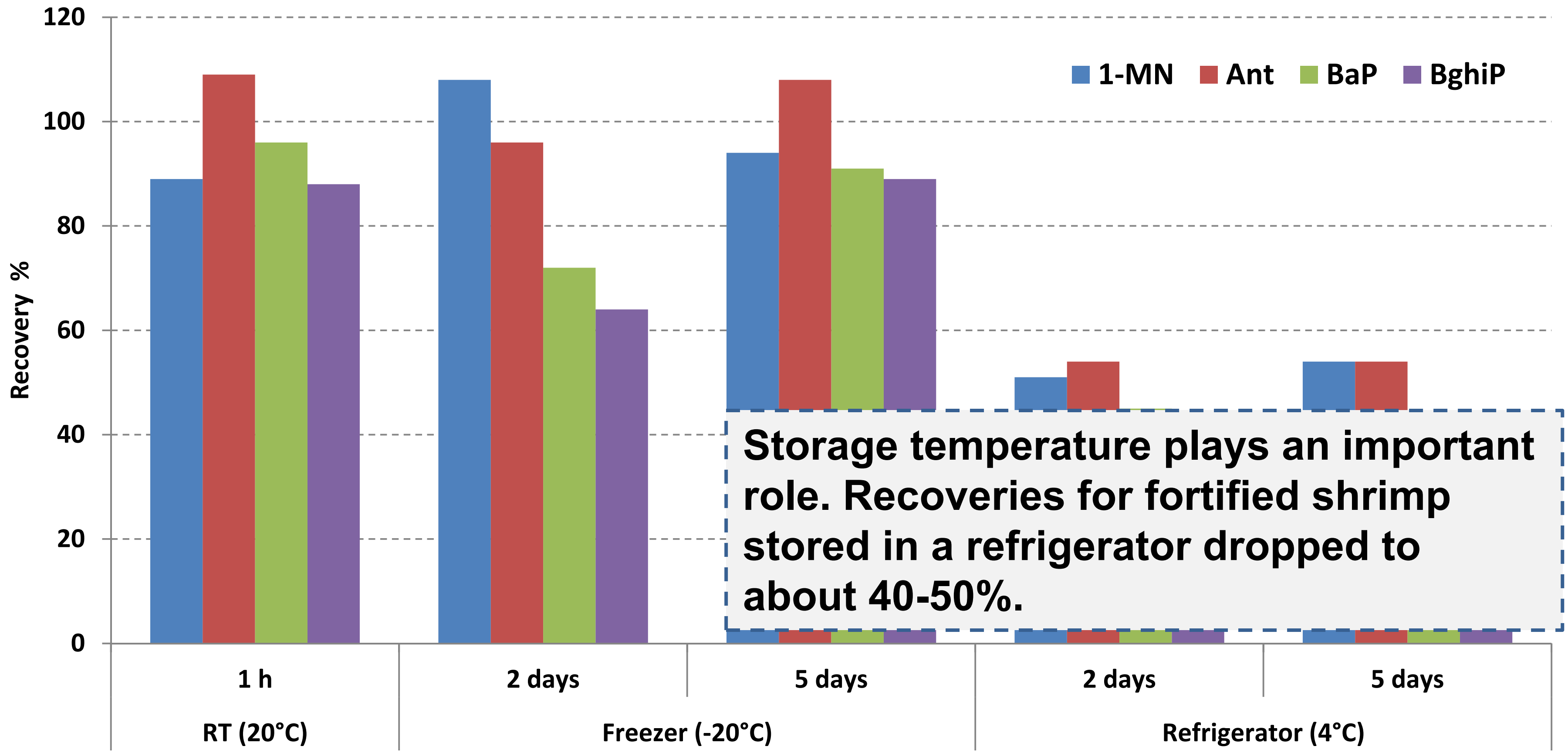

Homogenization using N_2
Extraction temperature
Water amount used for extraction
Extraction time
Storage temperature
Sample preparation – peeled x unpeeled shrimps

The influence of sample preparation on recovery of PAHs in spiked shrimps



Recoveries around 100% were obtained using the original method for all tested PAHs when both peeled and unpeeled shrimp were processed by blender only

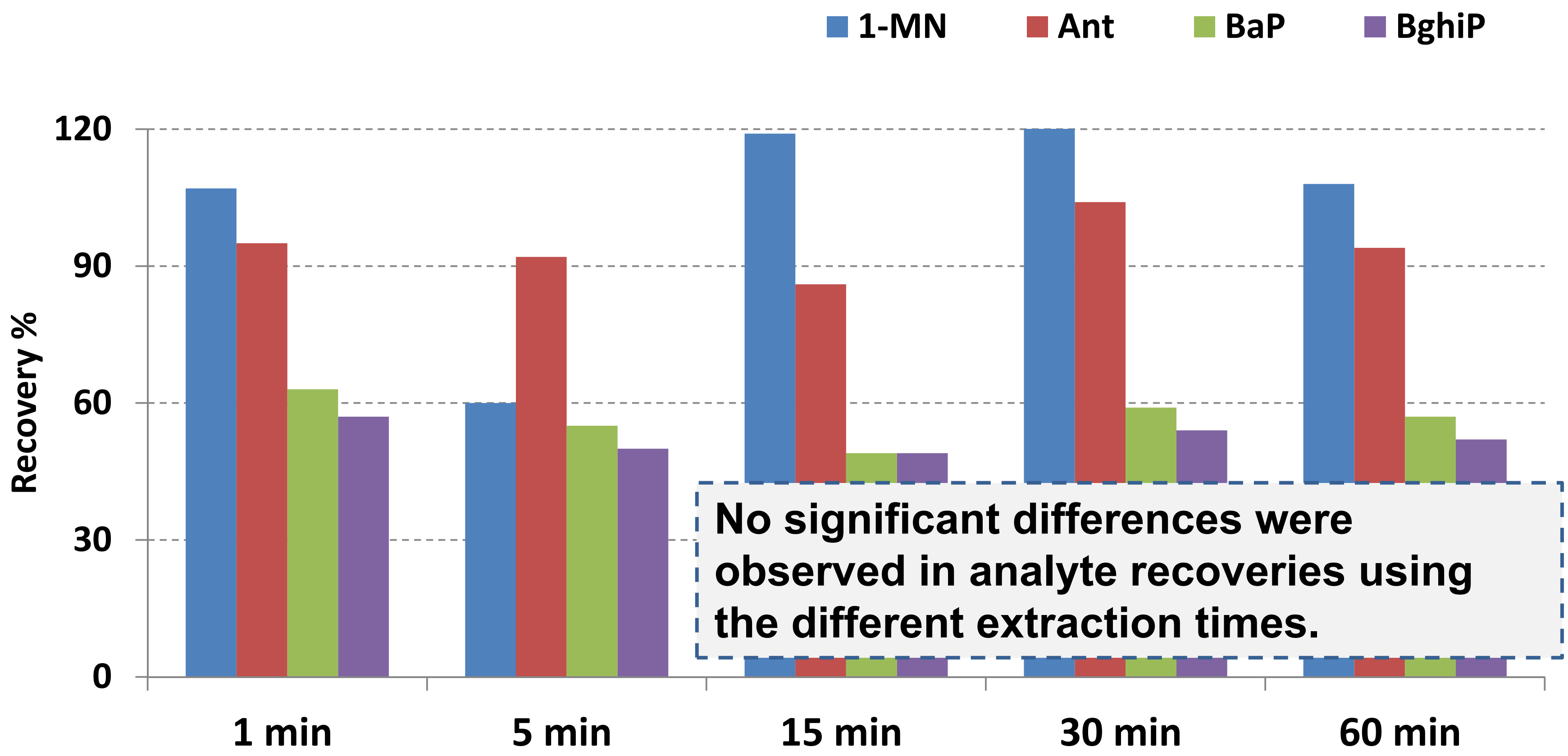
The influence of storage temperature on recovery of PAHs in spiked shrimps



Storage temperature plays an important role. Recoveries for fortified shrimp stored in a refrigerator dropped to about 40-50%.

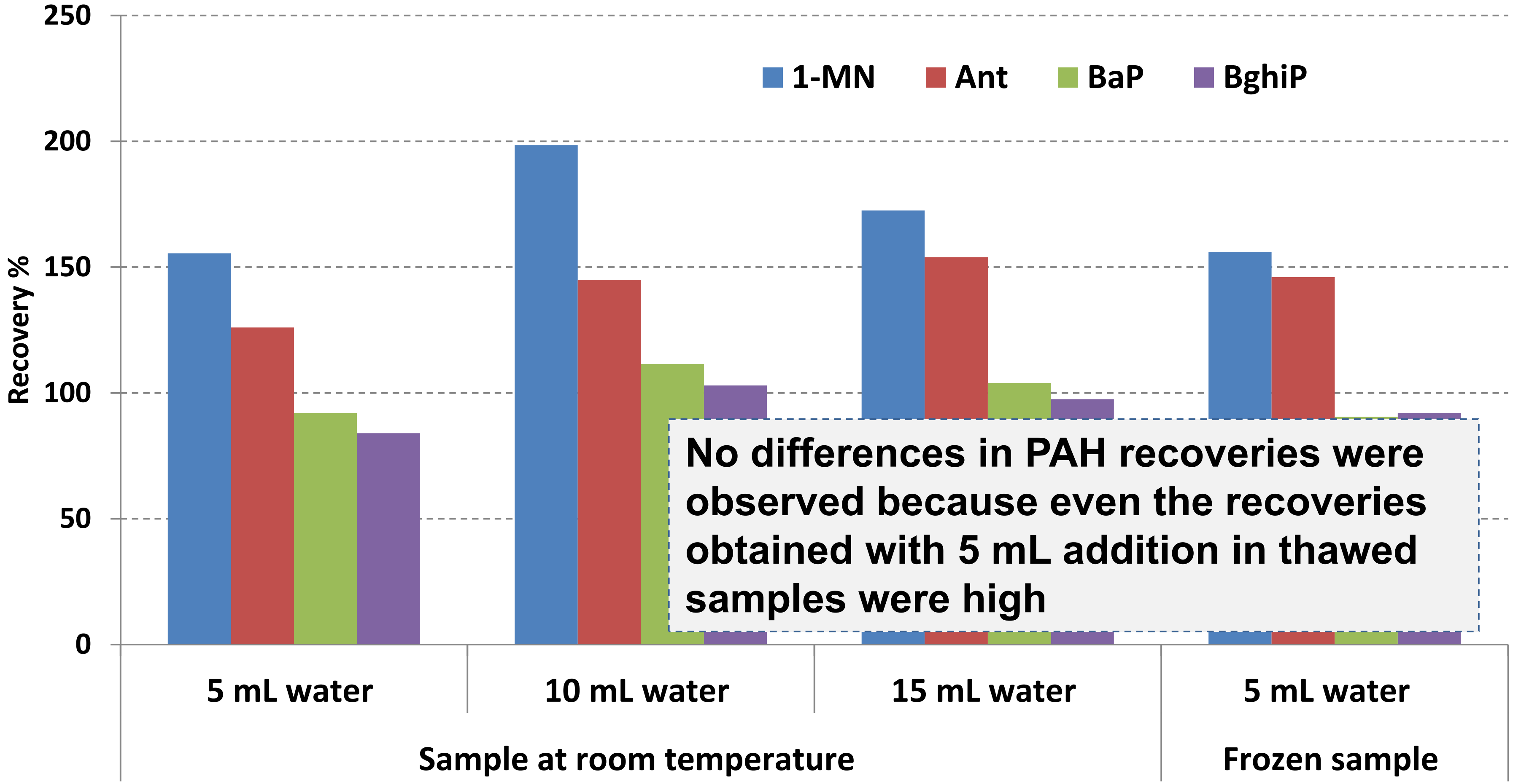
The influence of extraction time on recovery of PAHs in spiked shrimps

Spiked peeled and blended shrimps



No significant differences were observed in analyte recoveries using the different extraction times.

The influence of water amount on recovery of PAHs in spiked shrimps



Shrimp represent a more viscous matrix, for which a larger volume (10 mL) addition could improve shaking and matrix-solvent interaction

Optimized shrimp samples preparation

- processed by blender only
- spiked before extraction in laboratory
- 10 ml of water used for extraction
- 1 min of shaking for extraction
- Sample store temperature ≤ - 20°C

PAH	Mean	RSD (%)
1,7-DMP	99	12
1-MN	95	12
1-MP	94	11
2,6-DMN	94	12
3-MC	101	11
Ant	90	12
BaA	92	10
BaP	92	9
BbF	92	9
BghiP	88	8
BkF	95	10
Chr	94	10
DBahA	93	10
Fln	91	11
Flt	[illegible]	[illegible]
Icd	[illegible]	[illegible]
Na	[illegible]	[illegible]
Phe	91	10
Pyr	92	11

Recoveries : 88 – 101 %

Repeatability: 8 – 13 %

(2) **Test sample analysis**

- 3 matrices: mussels, oysters, shrimps
- total of 5 different levels of BaP (2 - 50 µg/kg)
- other studied PAHs at varying levels from 2 to 250 µg/kg that mimic typical PAH patterns
- each matrix fortified at 3 different concentration levels in duplicate

Preliminary results:

	n	Recovery %	RSD %
Mussels	6	82–111	1–6
Oysters	6	48–106	2–13
Shrimps	6	78–117	3–10

- Lower recoveries of BaP and Ant from oyster samples stored at -20°C

Conclusions

(1) Laboratory qualification

- **15 laboratories started** the collaboration study
- The problems with the recovery of the spiked shrimps were resolved
- **10 laboratories** have been **qualified** and started the actual study (analysis of the test samples)

(2) Test sample analysis

- **Preliminary results:**

Mussels, oysters and shrimps:

Recovery: 48 – 117%
Repeatability: 1 – 13%

Lower recoveries of BaP and Ant from oyster samples stored at -20°C

Thank you for your attention…