Advanced Analytical Technologies for Analyzing Environmental Matrixes Contaminated with Petroleum Hydrocarbons

Sample Preparations

Chemistries and Supplies Division

Joan M. Stevens, Ph.D.
Sample Preparation Application Chemist

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Outline

- Legislation and Established procedures
  - FDA and NOAA Legislation
    - Parameters used to measure contamination
    - Sample preparation procedure
- QuEChERS sample preparation approach
  - Sample preparation
  - Analysis

References

”Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants”, Sloan, C.A., Brown, D.W., Pearce, R.W., Boyer, R.H., Bolton, J.L., Burrows, D.G., Herman, D.P., and Krahn, M.M.U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-59, 47 pp., 2004

“Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry Results for Re-Opening Oil-Impacted Areas Closed to Seafood Harvesting”, 2010_0529_NOAA Opening Protocol Final, 8 pp., 2010

“The Analysis of Poly Aromatic Hydrocarbons in Biota and Sediment Extracts Using GC-MS/MS with the Agilent 7000A GC-QQQ System” Chris Sandy, Agilent Technologies UK, 44 pp, Oct 2009

“GC/MS Analysis of European Union (EU) Priority Polycyclic Aromatic Hydrocarbons (PAHs) using an Agilent J&W DB-EUPAH GC Column with a Column Performance Comparison”, Doris Smith and Ken Lynam, Agilent Technologies, USA, 6 pp, pub 5990-4883EN, Oct 2009.

Legislative Authorities

US Food & Drug Administration (FDA)

- Operates a mandatory safety program for all fish and fishery products
  - Under Federal Food, Drug & Cosmetic Act and related regulations

National Oceanic and Atmospheric Administration (NOAA)

- Legislative authority to open & close federal waters for seafood harvest
  - Operates the Seafood Inspection Program

Established Procedures

After an oil spill occurrence

- Federal and State agencies are faced with the issue of determining when seafood from the previously contaminated area may be safe for harvest and human consumption
  - NOAA and FDA work with other federal & state agencies to protect consumers from adulterated and unsafe seafood
    - Minimize undue economic burden on impacted seafood industries

Established Procedures

Once oil or chemical contamination visually observed on the surface

- Recommend fishery closure until free of sheen and analytical testing has occurred: establishing seafood wholesome and safe

  – Testing includes: Organoleptic analysis of products (sensory testing)
  Chemical analysis

  – Fishery closure areas: buffer zone around contaminated waters to account for uncertainty

Oil Contamination : Risks Assessments

Oil contamination presents 2 kinds of risks:

- Presence of petroleum tainted seafood rendering unfit for human consumption
- Presence of polycyclic aromatic hydrocarbons (PAHs) that are chemical hazards
  - Established FDA levels
    - Persistence
    - Potential Toxicity
    - Carcinogenic effects
  - FDA's "List of 9 PAHs"
    - Including their 16 alkylated homologues

FDA's " List of Nine-PAHs"

Criteria for Re-opening Areas Closed from Oil Spills Based on 160 g/day Seafood Consumption and Concentrations of Chemical Contaminants in Seafood

Chemical[1]	Level of Concern (ppm)	Basis[2]
Napthalene	20	EPA RfD; 70 kg bw; 160 g/day consumption
Fluorene	20	EPA RfD; 70 kg bw; 160 g/day consumption
Anthracene/phenanthrene	150	EPA RfD; 70 kg bw; 160 g/day consumption
Fluoranthene	0.15	10^{-6} Cancer risk estimate = 0.02B(a)P equivalency
Pyrene	0.025	10^{-6} Cancer risk estimate = 0.13B(a)P equivalency
Benz(a)anthracene	0.2	10^{-6} Cancer risk estimate = 0.014B(a)P equivalency
Chrysene	0.25	10^{-6} Cancer risk estimate = 0.013B(a)P equivalency
Benzo(a)pyrene	0.003	10^{-6} Cancer risk estimate = (34ng/p/d)(70/5yr)/160 g seafood/p/d

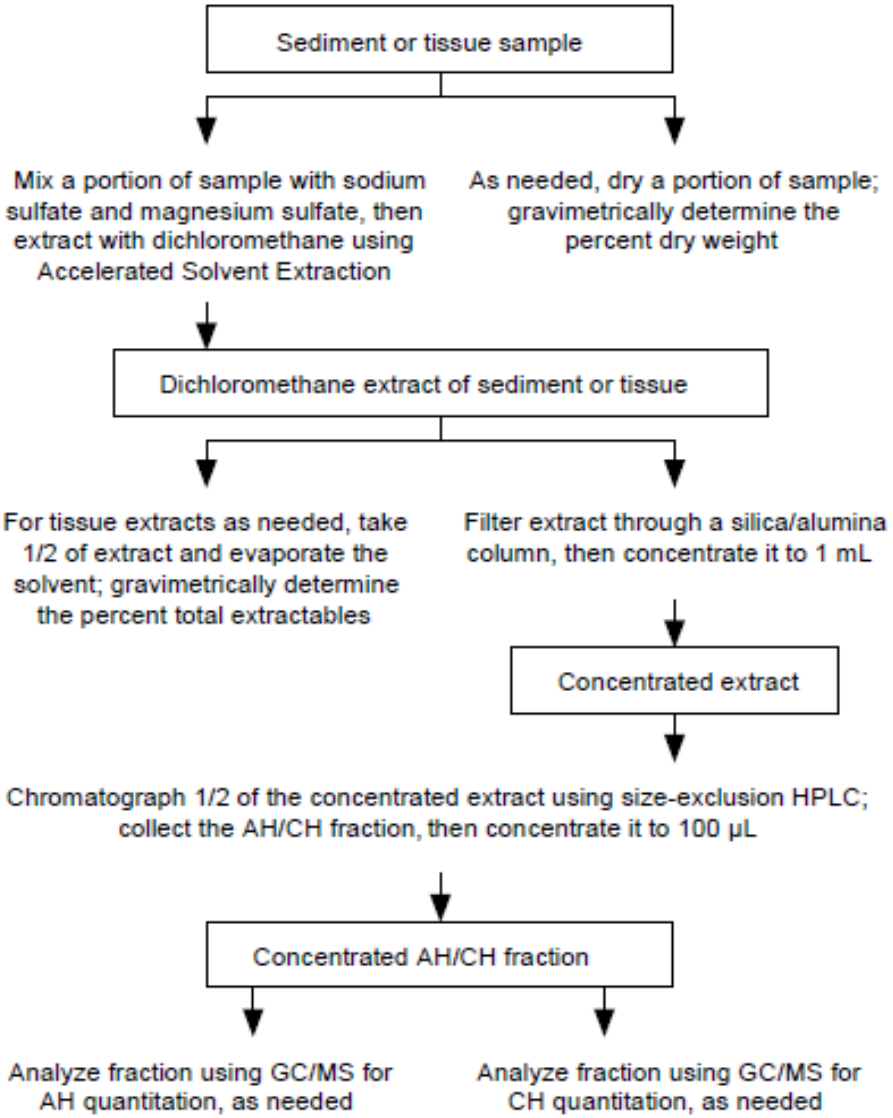
[1] Includes alkylated homologues, specifically C-1, C-2, C-3, C-4 napthalenes; C-1, C-2, C-3 fluorenes; C-1, C-2, C-3 anthracenes/phenanthracenes; C-1, C-2 pyrenes

List of 29 PAHs

Naphthalene
1-Methylnaphthalene
2-Methylnaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene
Acenaphthene
2,3,5-Trimethylnaphthalene
Fluorene
Dibenzothiophene
Phenanthrene
Anthracene
1-Methylphenanthrene
Fluoranthene
Pyrene
Retene
Benz[*a*]anthracene
Chrysene + Triphenylene *
Benzo[*b*]fluoranthene
Benzo[*j*]fluoranthene + Benzo[*k*]fluoranthene *
Benzo[*e*]pyrene
Benzo[*a*]pyrene
Perylene
Indeno[1,2,3-*cd*]pyrene
Dibenz[*a,h*]anthracene + Dibenz[*a,c*]anthracene *
Benzo[*ghi*]perylene

* These analytes are quantitated and reported as the sum of their concentrations because they co-elute during GC/MS analysis.

NOAA Sample Preparation Procedure



Alternative Procedure: QuEChERS

QuEChERS: **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**obust and **S**afe

- Initial purpose/validation was to determine pesticides in fruit and vegetables

- "QuEChERS works so well with pesticides can it work for other compound extracts"
- Advancements in QuEChERS has offered PAH determination in seafood

  – Why: Because of its "NAME"
    - Takes 10 minutes versus overnight for the NOAA method
      – Less time, Less solvent, Less glassware, Less cost, Less solvent disposal, Less subject to error, No chlorinated solvent

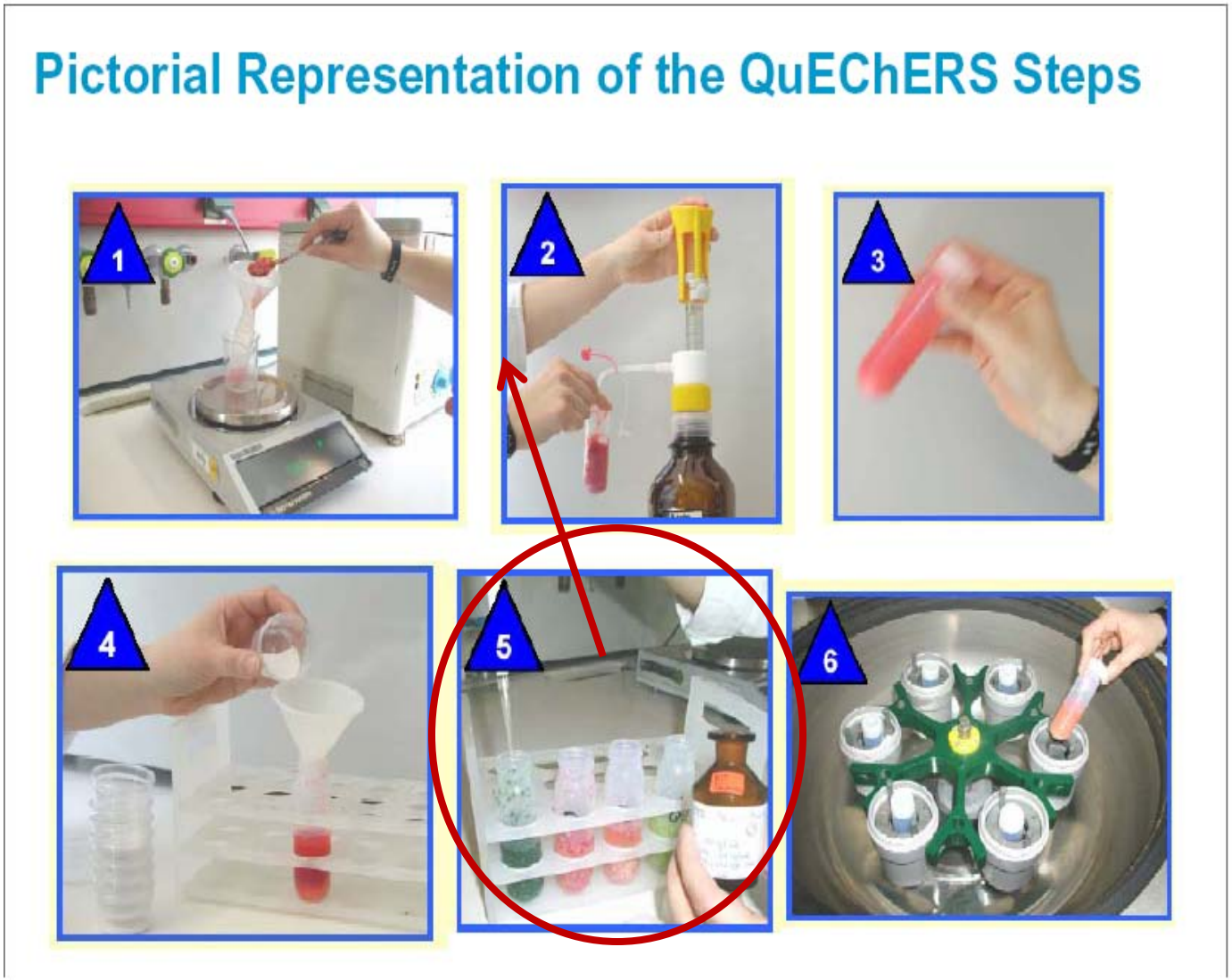
  – So let's take a look at QuEChERS

QuEChERS Procedure:

Chop then

Freeze

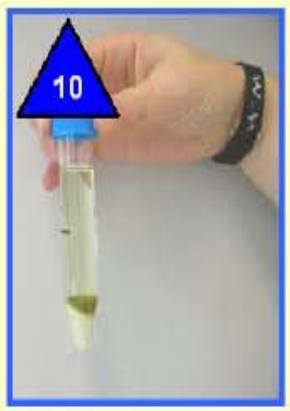
First Step – Extraction/Partitioning



1) Weigh sample
2) Add Ceramic Homogenizers
2) **Add standards**
3) **Vortex**
4) Add ACN (1% AA)
5) Vortex
6) Add salts
7) Shake 1 min
8) Centrifuge

Second Step – Dispersive SPE

1) Choose d-SPE
2) Transfer volume
3) Vortex 1 min
4) Centrifuge
5) Analyze



QuEChERS and d-SPE Sample Preparation Workflow

Weigh 3 g fish sample (+/- 0.1g) in 50 mL centrifuge tube

Add Surrogate/IS solution, and QC spike solution if necessary, Vortex 1 min

Add 12 mL of DI water and 2 ceramic bars to the sample (Agilent part #5982-9313), Vortex 1 min

Add 15 mL of ACN containing 1%HAc

Vortex 1 min

Add Agilent SampliQ QuEChERS AOAC extraction salt packet
(Agilent part #5982-5755)

Cap and shake vigorously for 1 min on Geno/Grinder at1500 rpm

Centrifuge at 4000 rpm for 5 min

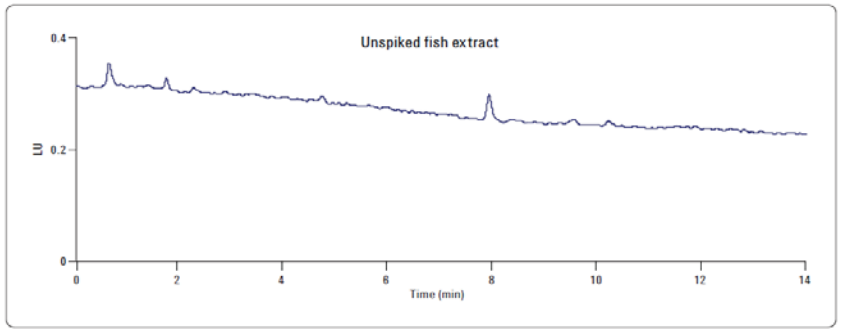
Transfer 1 mL of upper ACN layer to
SampliQ AOAC Fatty dispersive SPE 2 ml tube (Agilent part # 5982-5122)
Or 8 mL to SampliQ AOAC Fatty dispersive SPE 15 mL tube (Agilent part # 5982-5158)

Vortex 1 min, Centrifuge at 13000 rpm for 2 min for 2 mL tubes,
Or at 4000 rpm for 5 min for 15 mL tubes

Transfer 500 µL extract to autosampler vial

Analyze by LC/FLD or GC/MS

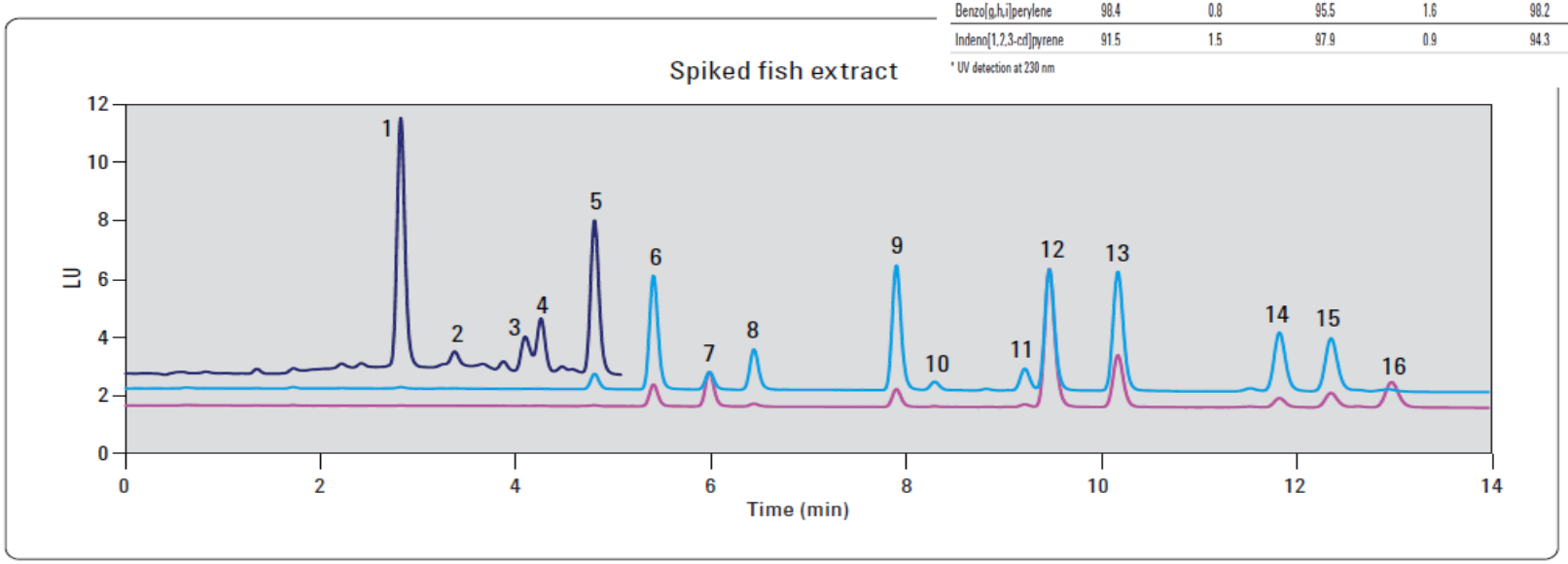
PAH Analysis by LC/FLD



Recoveries and RSDs for the Sixteen Polycyclic Aromatic Hydrocarbons in Fish Sample (n = 6)

PAH	Level of spiking (ng/g) (n = 6)					
	1		2		3	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	94.7	1.4	97.9	1.1	93.8	1.4
*Acenaphthylene	87.8	1.7	96.3	1.2	85.6	0.8
Acenaphthene	92.1	1.5	93.0	1.8	96.7	0.8
Fluorene	98.1	1.5	89.9	1.0	97.2	0.9
Phenanthrene	90.6	0.9	93.8	0.8	83.1	1.7
Anthracene	96.7	1.0	87.6	0.8	92.1	0.6
Fluoranthene	83.4	1.3	93.9	1.5	95.9	1.2
Pyrene	93.5	1.8	86.1	1.3	95.0	1.4
1,2-Benzanthracene	94.5	1.3	89.6	1.6	94.9	1.0
Chrysene	101.0	1.4	97.8	1.7	87.2	1.6
Benzo[e]pyrene	88.8	1.5	85.2	1.9	95.0	1.4
Benz[e]acenaphthylene	95.5	0.7	92.7	0.7	89.2	0.9
Benzo[k]fluoranthene	93.5	0.8	94.6	0.9	98.9	0.8
Dibenzo[a,h]anthracene	88.2	0.9	97.3	1.1	97.1	0.6
Benzo[g,h,i]perylene	98.4	0.8	95.5	1.6	98.2	0.7
Indeno[1.2.3-cd]pyrene	91.5	1.5	97.9	0.9	94.3	0.7

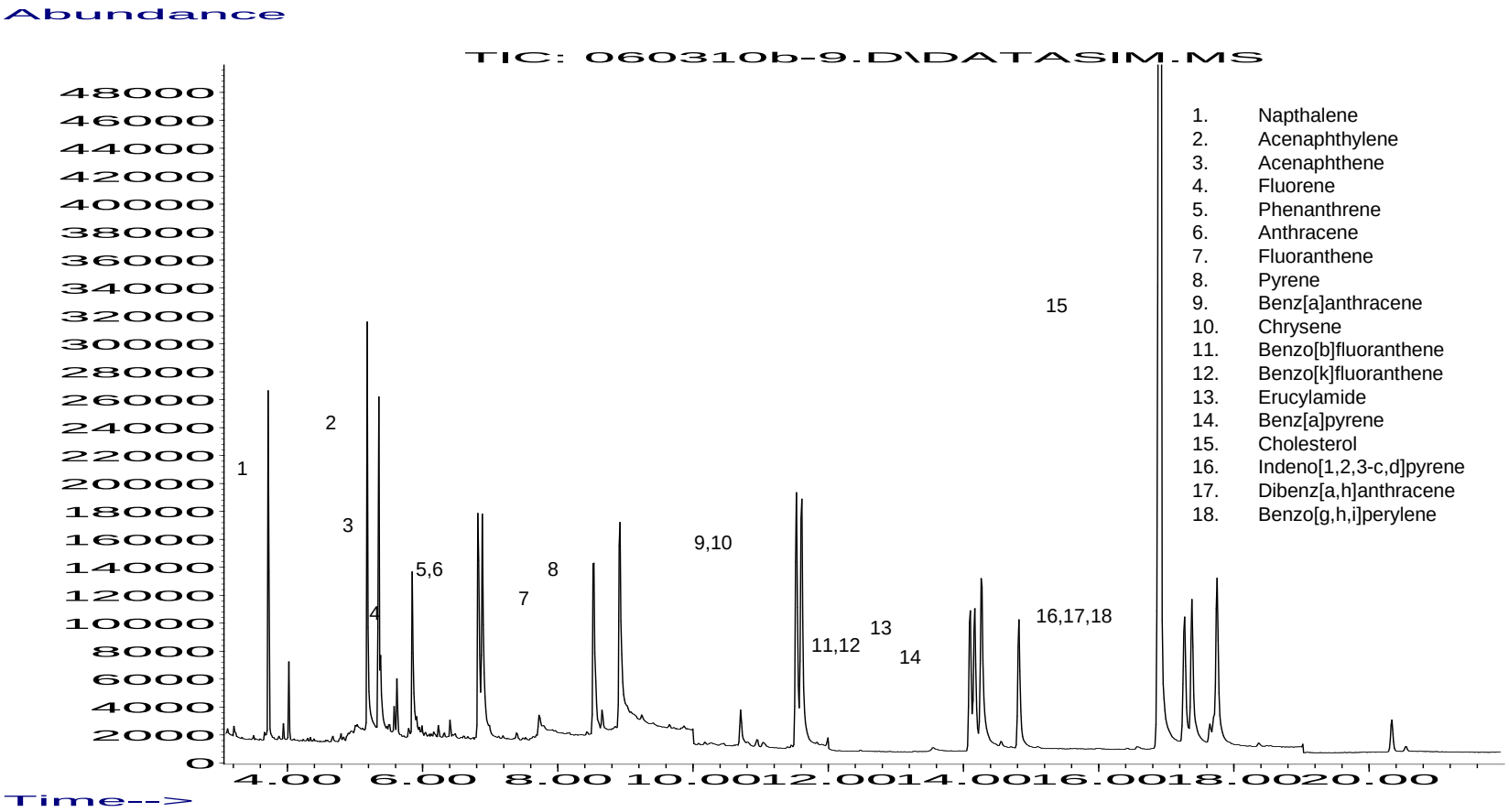
* UV detection at 230 nm



Overlay HPLC – FLD chromatograms of the spiked fish sample containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The spiking level for this sample was level 1 The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used

Agilent Technologies

125 ppb EPA PAHs extracted from Swai fish using QuEChERS
DB-5ms 20m 0.18mm 0.18µm
GC/MS SIM TIC



1. Napthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Erucylamide
14. Benz[a]pyrene
15. Cholesterol
16. Indeno[1,2,3-c,d]pyrene
17. Dibenz[a,h]anthracene
18. Benzo[g,h,i]perylene

Conclusion

– QuEChERS: offers a simple sample preparation approach to the extraction and analysis of PAHs in finfish and shellfish

– The simplicity and quickness associated with QuEChERS sample preparation allows multitudes of samples to be processes per day versus weeks