# Development of modified QuEChERS extraction methods for the analysis of PAHs in high fat content fish and smoked salmon

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**Rationale**:

### Chemicals of Concern

Polycylic aromatic hydrocarbons (PAHs) are a class of pollutants that can contaminate biotic tissue exposed to pyro- and/or petrogenic processes (i.e biomass combustion and oil spills). They are composed principally of carbon and hydrogen atoms arranged into greater than two aromatic rings and have log$K_{OW}$s ranging from 3 – 7. It is known that some PAHs, such as benzo[*a*]pyrene, posses genotoxic, mutagenic and carcinogenic properties. As such, user-friendly analytical methods that allow for simple, efficient and robust quantification of PAHs are of great interest.

### Analytical Methods

Sample preparation using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approaches are becoming increasingly popular for chemical extraction and clean-up from fruits and vegetables. Originally developed for pesticides, QuEChERS methods traditionally involve sample extraction into acetonitrile, subsequent liquid-liquid partitioning following addition of $MgSO_4$ and NaCl and dispersive SPE clean-up[a]. However, little information has been published on the applicability of these techniques for analyzing highly hydrophobic contaminants in high fat content bio-matricies such as smoked meats or oiled organisms.

**OBJECTIVE**: Develop and validate a sensitive QuEChERS sample preparation method for identifying and quantifying PAHs in high fat bio-matrices

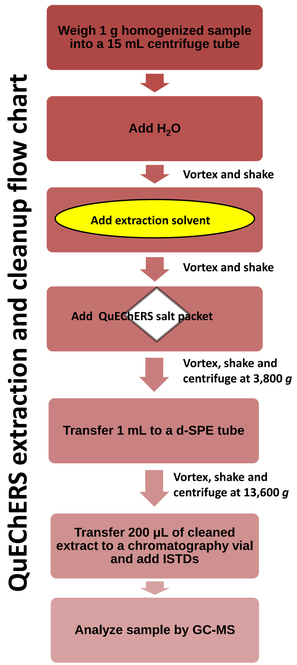
## QuEChERs Sample Preparation Methodology

**Samples:** Commercially available smoked salmon with 3, 8 and 11 % fat, as reported in product nutrition fact labels, were homogenized via freeze fracture with liquid $N_2$ in a food processor.

**Extraction:** Solid-liquid extraction into an organic solvent, iquid-liquid partitioning with QuEChERS EN or AOAC extraction salts and centrifugation

**Clean-up:** QuEChERS fatty sample d-SPE tubes and centrifugation

**Chemical analysis**: GC-MS equipped with a DB-5MS column , inlet temp: 300° C, flow rate: 1 mL/min, ionization: electron impact (70 eV), SIM mode, total run time - 36 min.

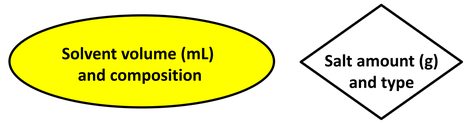



### Quantitative PAHs

| Compound | DB5 $R_t$ (min) | Target compound SIM ions (m/z) | | $R^2$ | †MDL (µg kg$^{-1}$) | FDA levels of concern (µg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| | | Quant | Confirm | | | |
| *Perylene D-12* | *25.72* | *264* | *260, 265* | *Internal standard* | | |
| Naphthalene | 8.77 | 128 | 127, 129 | 0.999 | 2 | 32,700 |
| 2-Methylnaphthalene | 10.30 | 142 | 141, 115 | 0.999 | 2 | - |
| 1-Methylnaphthalene | 10.52 | 142 | 141, 115 | 0.999 | 2 | - |
| 1,6-Dimethylnaphthalene | 11.97 | 156 | 141, 153 | 0.999 | 2 | - |
| 1,2-Dimethylnaphthalene | 12.35 | 141 | 156, 115 | 0.999 | 2 | - |
| Acenaphthylene | 12.34 | 152 | 151, 150 | 0.999 | 2 | - |
| Acenaphthene | 12.75 | 153 | 154, 152 | 0.999 | 2 | - |
| Fluorene | 13.96 | 166 | 165, 167 | 0.999 | 2 | 65,300 |
| Dibenzothiophene | 15.91 | 184 | 139, 185 | 0.999 | 2 | - |
| Phenanthrene | 16.21 | 178 | 176, 179 | 0.999 | 10 | 490,000 |
| Anthracene | 16.33 | 178 | 176, 179 | 0.996 | 10 | - |
| 2-Methylphenanthrene | 17.42 | 192 | 191, 165 | 0.998 | 10 | - |
| 2-Methylanthracene | 17.53 | 192 | 191, 165 | 0.992 | 10 | - |
| 1-Methylphenanthrene | 17.67 | 192 | 191, 165 | 0.999 | 10 | - |
| 9-Methylanthracene | 18.00 | 192 | 191, 165 | 0.999 | 10 | - |
| 3,6-Dimethylphenanthrene | 18.43 | 206 | 191, 205 | 0.999 | 2 | - |
| Fluoranthene | 19.01 | 202 | 203, 200 | 0.999 | 2 | 65,300 |
| 2,3-Dimethylanthracene | 19.09 | 206 | 191, 205 | 0.996 | 10 | - |
| 9,10-Dimethylanthracene | 19.60 | 206 | 191, 205 | 0.998 | 2 | - |
| Pyrene | 19.53 | 202 | 200, 203 | 0.999 | 2 | 49,000 |
| Retene | 20.27 | 219 | 220, 234 | 0.999 | 2 | - |
| 1-Methylpyrene | 20.86 | 216 | 215, 217 | 0.999 | 2 | - |
| Benz(a)anthracene | 22.37 | 228 | 226, 229 | 0.983 | 10 | 350 |
| Chrysene | 22.45 | 228 | 226, 229 | 0.994 | 2 | 35,000 |
| 6-Methylchrysene | 23.49 | 242 | 241, 226 | 0.999 | 2 | - |
| Benzo(b)fluoranthene | 24.78 | 252 | 253, 250 | 0.994 | 2 | 350 |
| Benzo(k)fluoranthene | 24.85 | 252 | 253, 250 | 0.992 | 2 | 3,500 |
| Benzo(e)pyrene | 25.44 | 252 | 250, 253 | 0.999 | 2 | - |
| Benzo(a)pyrene | 25.57 | 252 | 253, 250 | 0.996 | 2 | 35 |
| *Indeno(1,2,3-c,d)pyrene D-12* | *28.78* | *288* | *284, 289* | *Internal standard* | | |
| Indeno(1,2,3-c,d)pyrene | 28.86 | 276 | 277, 274 | 0.997 | 2 | 350 |
| Dibenz(a,h)anthracene | 28.95 | 278 | 279, 276 | 0.998 | 10 | 35 |
| Benzo(ghi)perylene | 29.63 | 276 | 277, 274 | 0.999 | 2 | - |
| Dibenzo(a,l)pyrene | 33.91 | 302 | 300, 303 | 0.998 | 2 | - |

Table 1. Summary of PAH GC-MS instrumental details and comparison to FDA levels of concern for finfish. †MDL = IDL x 2, where IDL was assigned when PAH lowest abundance SIM confirmation ion S/N > 3 for standards prepared in iso-octane.

## Modified QuEChERS Experimental Design




| Extraction Method | Solvent volume (mL) and composition | Salt amount (g) and type |
|---|---|---|
| Traditional E1[b,c] | 5; 1% acetic acid/acetonitrile | 2.5; AOAC[†] |
| Traditional E2[d,e] | 2; acetonitrile | 1.3; EN[††] |
| Modified E3 | 2; 2:2:1 ethyl acetate, acetone, iso-octane | 1.3; AOAC |
| Modified E4 | 2; 2:2:1 ethyl acetate, acetone, iso-octane | 1.3; EN |

Table 2. Four QuEChERS methods compared on the basis of PAH extraction efficiency from smoked salmon. † AOAC salts – $MgSO_4$ and $NaC_2H_3O_2$; †† EN salts – $MgSO_4$, NaCl, $NaC_6H_7O_7$ and $Na_2C_6H_6O_8$. Extraction methods E1 and E2 are based on published methods[b,c,d,e].

## Modified -vs- Traditional QuEChERS Recovery Performance

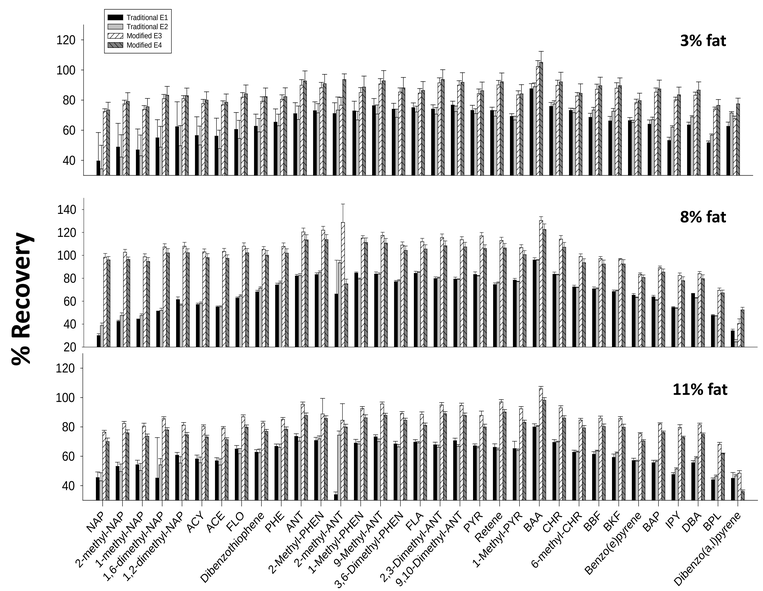



Figure 1. Average recovery of PAHs from smoked salmon (3 – 11% fat) generated with four different QuEChERS extraction methods (see Table 2 for details). Bars represent mean recoveries from four replicates ± SD.

## Modified QuEChERS -vs- Soxhlet and ASE

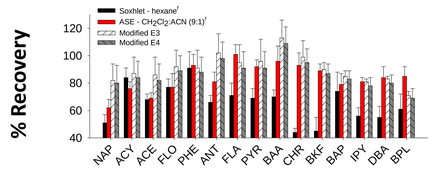



Figure 2. Comparison of PAH extraction performance (% recovery ± SD) for soxhlet, accelerated solvent extraction and QuEChERS methods (E3 and E4). Soxhlet and ASE data are from [f]Wang, 1999. E3 and E4 data represent mean PAH recovery across all three fish tested (*n* = 12/method).

## Determination of PAHs in smoked salmon by Modified QuEChERS

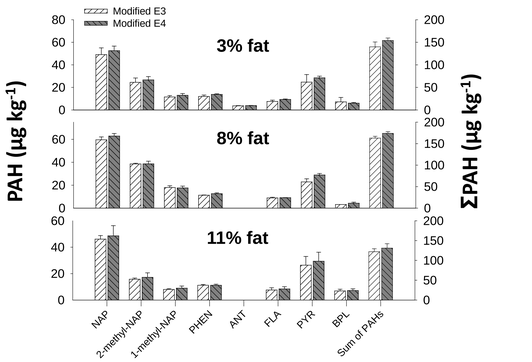



Figure 3. Comparison of PAH levels (mean ug kg$^{-1}$ wet weight ± SD, n = 3) measured in commercially available smoked salmon by two new QuEChERS extraction schemes (E3 and E4; see table 2 for details).

## Conclusions

- A modified QuEChERS analytical method has been developed and validated for PAHs in high fat bio-matrices (Table 1 & Fig 1 – 3).
- Greater recoveries of PAHs are achieved with 2:2:1 ethyl acetate, acetone and iso-octane than acetonitrile alone (Figure 2).
- The type of partitioning salt used does not impact recoveries (Figure 1 and 3).
- QuEChERS methods perform comparably to, or better than, Soxhlet and Accelerated Solvent Extraction (ASE) based methods (Figure 2).
- Method sensitivity is sufficient for regulatory agencies (Table 1).
- Future investigations will apply the method to assess the contribution of traditional salmon smoking to dietary PAH intake for a Native American tribe.

Acknowledgements: Thank you to Wendy Hillwalker, Ricky Scott and Kristin Pierre for helping to prepare and conduct extraction experiments. Funding for this research is from Grant # P42ES016465 of the Super Fund Research project.

References: [a]Anastassiades, M. (2003) *Journal of AOAC international*, 86, 412 – 431. [b]Feyerherm, F. (2010) Agilent Technologiies. *e-seminar*. [c]Smith, D. (2010) Agilent Technologies. *Application note*. [d]Cochran, J.(2010) Restek Corp. *Florida Pesticide Residue Workshop (FPRW) Poster*. [e]Ramalhosa, M.J. (2009) *J. Sep. Sci*. 3529-3538. [f]Wang et al. (1999) *J. Agric. Food Chem*. 1062 – 1066.