QuEChERS Extraction Methodology in the Extraction and Determination of PAHs in Shellfish and Finfish from the Gulf Oil Crisis

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds included in the European Union and US Environmental Protection Agency (US EPA) priority pollutant list because of their mutagenic and carcinogenic properties. Excluding smokers and occupational vulnerable populations, most individuals are exposed to PAHs predominately from dietary sources. In the marine environment PAHs are bioavailable to marine species via the food chain, as water-borne compounds, and contaminated sediments. As lipophilic compounds that can easily cross lipid membranes and have potential to bioaccumulate in aquatic organisms.

The QuEChERS method has been widely applied in the analysis of pesticides in food since it was introduced by USDA scientists. In general, there are two major steps, extraction and dispersive-SPE cleanup. The first step in the method employs acetonitrile, excess salts and magnesium sulfate ($MgSO_4$) to induce a liquid-liquid partitioning while simultaneously salting out the water from the aqueous sample. After centrifugation, transfer an aliquot from the top organic layer, for further cleanup, to a dispersive solid phase extraction (d-SPE) tube. The d-SPE contains a combination of PSA (primary secondary amine) sorbent to remove organic acids from the extract, C18 to remove fats/lipids, and anhydrous $MgSO_4$ to remove any remaining water in the extract.

This application offers a method for the analysis of PAHs at trace levels in fish tissue with GC/MS, or GC/MS/MS. The sample preparation approach used here demonstrates substantial time savings when compared with more traditional techniques used in the NOAA method for PAH analysis. The relatively clean extracts obtained using the QuEChERS method lend themselves nicely to either liquid or gas phase analysis. Orthogonal results can readily be obtained from the same extracts using very different analytical techniques. Levels of detection substantially below levels of concern are achieved, with 70-100% average recoveries, and RSDs at 3% or lower on average.

Experimental

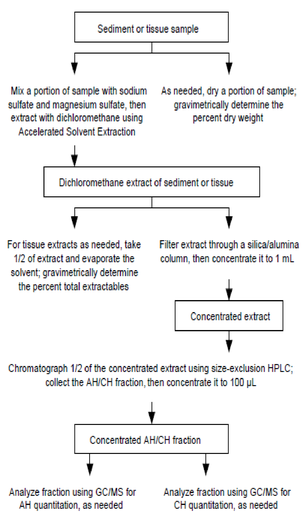
FDA: Established Levels of Concern

Table 1. FDA Levels of Concern established for the Horizon Deepwater oil spill, in parts per million (ppm)

Chemical[1]	Levels of Concern (ppm)		
	Shrimp and Crabs (13 g/day[2])	Oysters (12 g/day[2])	Finfish (49 g/day[2])
Naphthalene	123	133	32.7
Fluorene	246	267	65.3
Anthracene/Phenanthrene	1846	2000	490
Pyrene	185	200	49.0
Fluoranthene	246	267	65.3
Chrysene[3]	132	143	35.0
Benzo(k)fluoranthene[3]	13.2	14.3	3.5
Benzo(b)fluoranthene[3]	1.32	1.43	0.35
Benz[a]anthracene[3]	1.32	1.43	0.35
Indeno(1,2,3-cd)pyrene[3]	1.32	1.43	0.35
Dibenz[a,h]anthracene[3]	0.132	0.143	0.035
Benzo[a]pyrene[3]	0.132	0.143	0.035

1 Includes alkylated homologues, for example C-1, C-2, C-3, C-4 napthalenes, fluorenes, anthracenes, fluoranthenes, pyrenes and chrysenes. Alkylated homologues are assumed to have similar toxicities to the parent compounds.
2 Daily consumption level
3 Identified as a potential carcinogen by the FDA

NOAA Sample Preparation Procedure



Alternative Procedure: QuEChERS

QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe

- Initial purpose and validation was to determine pesticides in fruit and vegetables
- QuEChERS works well with pesticides can it work for the analysis of other compounds
- Advancements in QuEChERS has offered PAH determination in seafood
- The QuEChERS technique takes less time, solvent, no glassware or chlorinated solvents

QuEChERS Sample Preparation Workflow

Weigh 3 g of homogenized fish sample (0.1g) in 50 mL centrifuge tube

Add surrogate/IS solution, and QC spike solution if necessary, vortex 1min

Add 12 mL of DI water and 2 ceramic homogenizers to the sample (Agilent p/n 5982-9313)

Add 15mL of ACN containing 1% HAc, vortex 1 min

Add Agilent AOAC QuEChERS Extraction salt packet (Agilent p/n 5982-5755)

Cap and shake vigorously for 1min on GenoGrinder

Centrifuge @ 4000rpm for 5min

Transfer 1 mL of upper ACN layer to Agilent AOAC fatty matrix Dispersive-SPE 2 mL tube (Agilent p/n 5982-5122), or 8mL to Agilent fatty matrix AOAC Dispersive-SPE 15 mL tube (Agilent p/n 5982-5158)

Vortex 1min, centrifuge @ 13,000 rpm for 2 min for 2 mL tubes
Or @ 4000 rpm for 5 min for 15 mL tubes

Transfer 500 µL extract to autosampler vial,

Analyze by GC/MS or GC/MS/MS

Results and Discussion

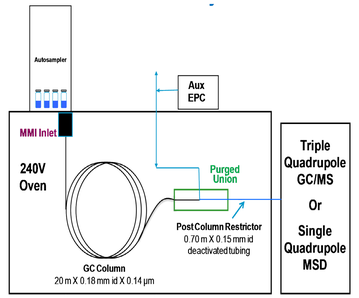


Figure 1: Schematic representation of the preconfigured PAH analysis system, equipped with an optimized GC column, MMI inlet and capillary flow technology backflushing

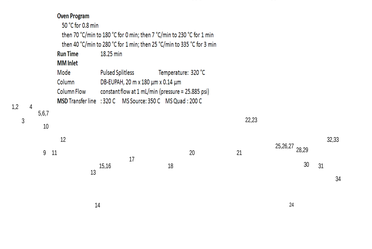
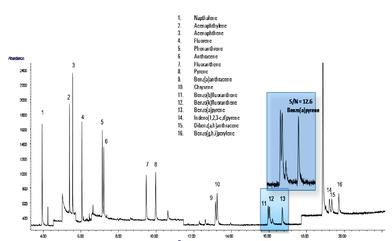


Figure 2: GC/MS analysis of PAH standards at 300 ppb for the 29 compounds listed by NOAA, using the Agilent 7890 GC and 5975C MSD in electron impact (EI) mode, equipped with capillary flow technology backflushing



GC/MS SIM Chromatogram of 10 ppb PAHs spiked in fish matrix blank extract

GC/MSD: 7890/5975B with purged ultimate union
Column: DB-5ms UI 20 m X 0.18 mm X 0.18 µm
Restrictor: Siltek 0.7m x 0.15mm ID (Sil 2)
MMInlet: 0.5µL, 320°C, splitless , purge flow 50mL/min at 0.8min gas saver 20mL/min at 2min
Carrier: Helium, 1.7mL/min const flow col 1
PCM 1=18 psi const pressure, col 2=3.8ml/min flow @ 50°C
Oven: 50°C (0.4 min) to 180°C (25°C/min) hold 1.5 min, 8°C/min to 280°C (0°C/min to 335°C (3.25 min)
Positive backflush 7 min @325°C
MSD: Transfer line 340°C Source 340°C Quad 150°C

Figure 3: GC/MS SIM chromatogram of 10 ppb spiked in fish matrix blank extract

Table 2: R^2 values from PAH calibration curves

RT	PAH	GC/MS/MS 1-1000 ppb in isooctane	GC/MS/MS 1-100 ppb in QuEChERS Extract	GC/MS 1-1000 ppb in isooctane
3.14	Napthalene	0.9998	0.9972	0.9997
3.43	1-methylnaphthalene	0.9998	0.9995	0.9998
3.53	2-methylnaphthalene	0.9999	0.9995	0.9996
3.76	Biphenyl	0.9998	0.9902	0.9998
3.78	2,6-dimethylnaphthalene	0.9998	0.9983	0.9999
4.24	Acenapthylene	0.9999	0.9994	0.9998
4.80	Acenapthene	0.9999	0.9999	0.9997
4.97	2,3,5- trimethylnaphthalene	0.9999	0.9998	0.9998
5.35	Fluorene	0.9999	0.9998	0.9998
6.48	Dibenzothiophene	0.9996	0.9989	0.9998
6.73	Phenanthrene	0.9997	0.9992	0.9999
6.79	Anthracene	0.9997	0.9985	0.9999
8.30	1-methylphenanthrene	0.9997	0.9996	0.9998
9.80	Fluoranthene	0.9960	0.9997	0.9998
10.68	Pyrene	0.9970	0.9998	0.9998
13.14	Benzo(a)anthracene	0.9930	0.9990	0.9998
13.29	Chrysene	0.9940	0.9997	0.9999
14.83	Benzo(b)fluoranthene	0.9997	0.9980	0.9987
14.86	Benzo(k)fluoranthene	0.9992	0.9983	0.9985
15.27	Benzo(e)pyrene	0.9999	0.9977	0.9987
15.33	Benzo(a)pyrene	0.9998	0.9971	0.9987
15.47	Perylene	0.9996	0.9977	0.9986
16.70	Indeno(1,2,3, -cd)pyrene	0.9997	0.9899	0.9996
16.69	Dibenz(a,h)anthracene	0.9980	0.9895	0.9996
17.23	Benzo(ghi)perylene	0.9888	0.9889	0.9991

•R2 values for linear fit of the calibration curves were obtained with the PAH standards, using either the Agilent 5975C MSD in EI mode (GC/MS), or the 7000A Triple Quadrupole GC/MS in SRM mode (GC/MS/MS). All calibration curves were determined using 7 concentration levels from either 1 to 1000 ppb (GC/MS/MS and GC/MS in isooctane) or 1 to 100 ppb (GC/MS/MS in QuEChERS extract). The GC/MS/MS in QuEChERS extract values, which were determined from an extract of fish at 1g/mL, are provided by Ralph Hindle at Vogon Laboratory Services Ltd.

Table 3: % Recovery values for the three samples spiked into mussels at 25 ppb with PAH standards*

PAH	Sample 1	Sample 2	Sample 3	Avge. % Recovery
Acenaphthylene	23.8	25.0	25.7	99
Acenaphthene	23.3	24.8	22.5	94
Fluorene	31.3	30.6	29.2	121
Phenanthrene	24.5	27.1	26.4	104
Anthracene	22.5	23.6	24.3	94
Fluoranthene	25.7	25.9	26.8	105
Pyrene	22.9	22.9	24.1	93
Benz[a]anthracene	29.2	27.9	29.9	116
Chrysene	24.0	23.4	24.3	96
Benzo[b]fluoranthene	22.0	23.1	23.6	92
Benzo[k]fluoranthene	20.7	21.9	22.2	86
Benzo[a]pyrene	27.0	29.5	31.7	117
Dibenz[a,h]anthracene	18.8	19.4	19.9	77
Indeno[1,2,3-cd]pyrene	17.3	17.9	18.7	72
Benzo[ghi]perylene	17.3	18.0	18.7	72

* Recovery values for selected PAHs, using either the Triple Quadrupole GC/MS in SRM mode or the MSD in SIM mode. The PAH standards were spiked into three separate mussel samples to give a concentration in the QuEChERS extract of 25 ppb.

Table 4: Comparison of signal-to-noise ratios for PAH standards analyzed at 1 ppb*

PAH	1 ppb in Isooctane GC/MS/MS	1 ppb in Isooctane GC/MS	25ppb in QuEChERS Extract GC/MS/MS	25ppb in QuEChERS Extract GC/MS
Naphthalene	36	23	---	---
Fluorene	8.0	7.2	112	92
Phenanthrene	6.7	8.8	121	69
Anthracene	6.8	5.7	100	60
Fluoranthene	8.0	5.3	88	43
Pyrene	6.3	4.6	105	39
Benz[a]anthracene	22	5.0	130	128
Chrysene	21	5.1	130	121
Benzo[a]pyrene	15	10	60	11

* Recovery measured at 1 ppb in isooctane, or at 25 ppb in QuEChERS extract of fish, using either SRM analysis on the Agilent 7000B Triple Quadrupole GC/MS (GC/MS/MS), or SIM mode on the 5975C single quadrupole MSD (GC/MS). While sensitivity is similar in the 2 systems with standards spiked into solvent, SRM analysis provides increased sensitivity in the mussel samples due to elimination of matrix interference. Signal to noise is measured peak-to-peak in this study.

Conclusions

- 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 per week
- A preconfigured analyzer can help your lab start running PAHs with higher productivity
- Backflushing will reduce cycle time and instrument maintenance for samples with matrix
- Signal-to-noise is about the same on a 5975C-Q using SIM compared to a 7000B-QQQ using MRM for clean samples
- The 7000B-QQQ analyzer can reach lower detection limits for PAHs, with greater confidence than the 5975C-Q for QuEChERS extracts for seafood