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part a

Alternative Procedure for Extraction and Analysis of PAHs in Seafood by QuEChERS-SBSE-GC-MS

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Abstract
The Deepwater Horizon oil rig explosion and the subsequent massive oil spill is expected to be the worst offshore oil catastrophe in United States history and is now beginning to impact fragile ecosystems, air and water quality, and food supplies.

Protecting humans from consuming foods contaminated with oil, while minimizing economical impacts for fisheries, is presenting several challenges. The current protocol for screening seafood harvested from the gulf is organoleptic testing followed by polycyclic aromatic hydrocarbons (PAHs) testing using NOAA Method 2004. It is generally thought that improved testing strategies will be needed to meet state screening requirements. A recent EPA Region 7 study has shown that Stir Bar Sorptive Extraction (SBSE) is an effective and fast technique for trace PAH determination in water. The goal of this study is to determine if using a QuEChERS solvent extraction in conjunction with SBSE can meet regulatory limits of detection and requirements established for precision and accuracy. Preliminary data show linear calibration for PAHs from 1-250 ng/g tissue in matrix matched extracts. Triplicate analyses estimates recoveries at 107 % ± 5 % RSD in fish tissue spiked with 2.5 or 25 ng/g PAH standards. It is also estimated that 40-60 homogenized samples/ analyst/day can be analyzed using this improved method. Further, validation studies are underway to assess the full potential of this method

Principle

This method uses a QuEChERS (quick, easy, cheap, effective, rugged, and safe) solvent extraction in conjunction with Stir Bar Sorptive Extraction (SBSE) to meet regulatory limits of detection and requirements established for precision and accuracy for determination of PAHs in seafood tissue.

The QuEChERS method uses a single-step acetonitrile (ACN) extraction and liquid–liquid partitioning based on salting out from the water in the sample using $MgSO_4$. The original QuEChERS procedure for pesticides used dispersive-solid-phase extraction (dSPE) cleanup to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and $MgSO_4$. However, this cleanup step provides no additional concentration factor making it difficult to achieve detection limits meeting the current requirements for PAH analysis. The procedure described herein uses SBSE as a combined cleanup and concentration step, eliminating organic acids and other polar and high molecular weight matrix components and providing a substantial concentration factor to easily achieve the regulatory detection limits.

In brief, 3 g of a homogenized seafood tissue sample in water is extracted with ACN in a 50 mL centrifuge tube followed by addition of 6.0 g $MgSO_4$ and 1.5 g sodium acetate which is shaken and centrifuged. A portion of the ACN extract (upper layer) is added to a 10 mL vial along with 4 mL 0.1 M $NaHCO_3$ and a GERSTEL Twister™ stir bar that is used to extract and concentrate the PAHs. The Twister stir bar is transferred to a thermal desorption tube in an autosampler tray for analysis by gas chromatography/mass spectrometry (GC/MS).

Experimental

Instrumentation. Analyses were performed on a 7890 GC equipped with a 5975C inert XL MSD with triple axis detector (Agilent Technologies), PTV inlet (CIS 4, GERSTEL), TDU Thermal Desorption Unit and MPS 2 autosampler (GERSTEL).

Analysis conditions.

TDU:	splitless
	40°C, 720°C/min, 300°C (5 min);
PTV:	0.2 min solvent vent (100 mL/min)
	split 10:1
	-120°C (0.2 min), 12°C/sec,
	300°C (3 min)
GC Oven:	30 m Rxi®-5Sil MS (Restek)
	d_i = 0.25 mm d_f = 0.25 µm
	He, constant flow (1 mL/min)
	60°C (1 min), 15°C/min, 325°C (3 min)

Results and Discussion

Seafood sample matrices tested: croaker, clam, red snapper, mussel, shrimp, sole, oyster, and scallop.

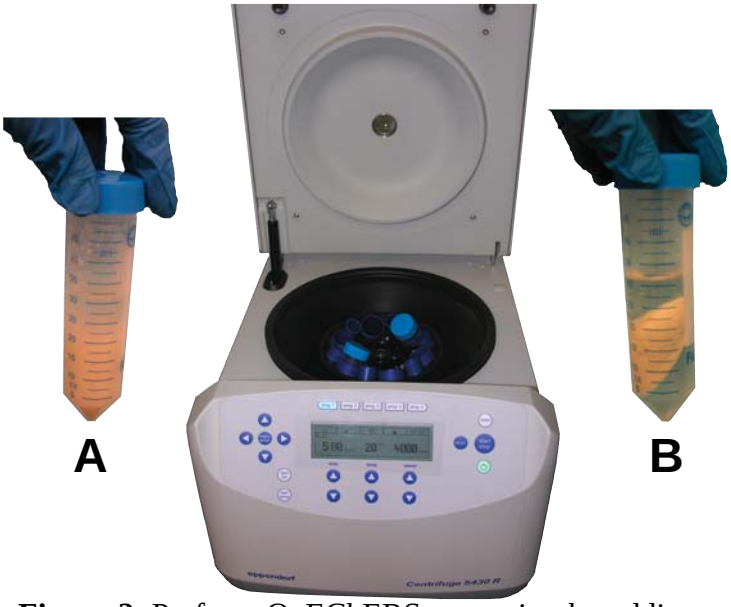


Figure 2. Perform QuEChERS extraction by adding water, acetonitrile and salt (A) and centrifuge to separate layers (B).

Figure 1. Sample is frozen and homogenized.

Figure 3. Dilute 1 mL acetonitrile extract with 0.1 M $NaHCO_3$ and perform SBSE.

Figure 4. Desorb GERSTEL Twister using Thermal Desorption Unit.

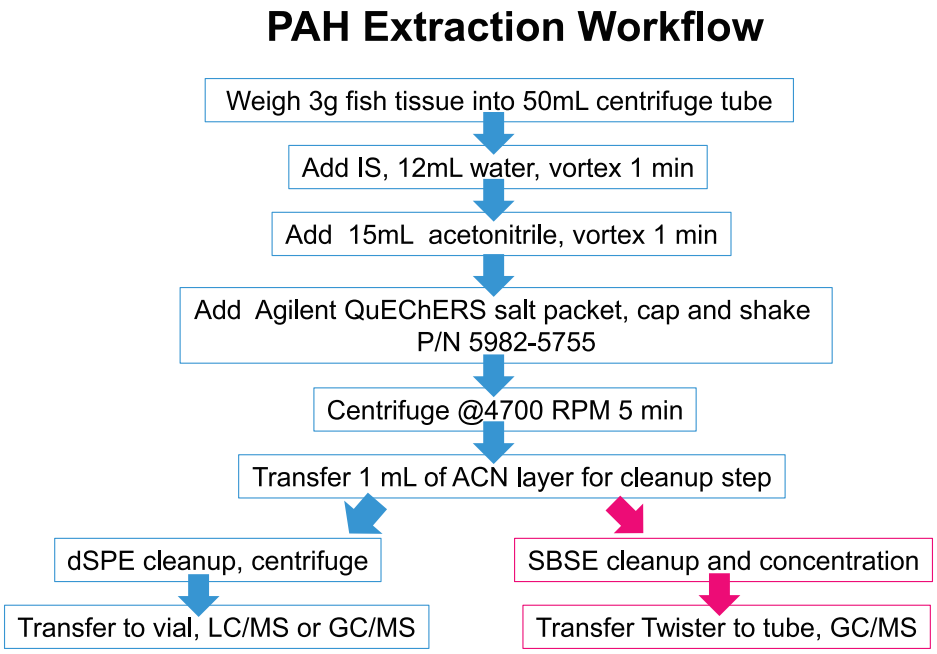


Figure 5. Comparison of workflow for conventional QuEChERS and QuEChERS/SBSE method.

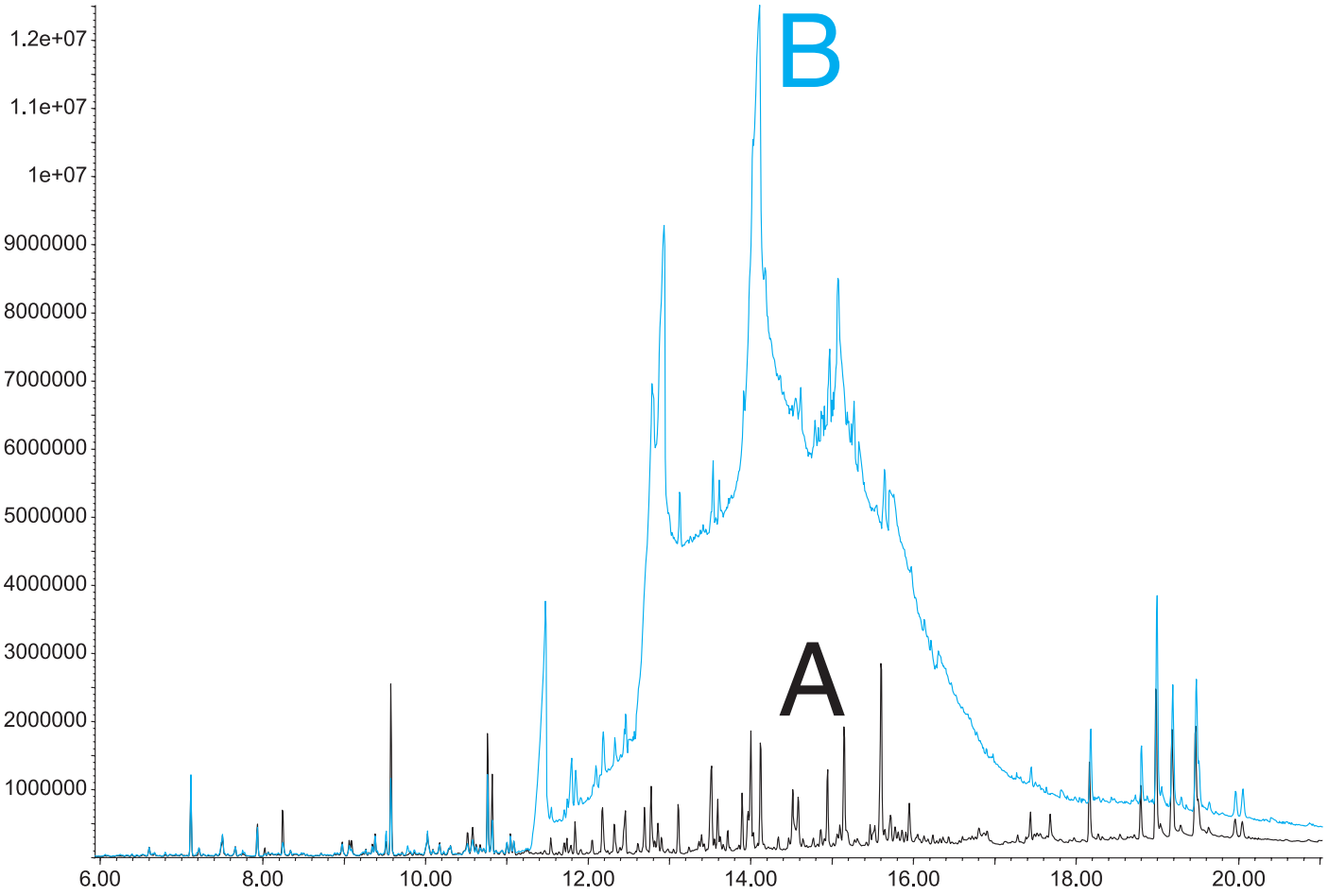


Figure 6. SBSE performed with $NaHCO_3$ (A) instead of water (B) significantly reduces matrix interference.

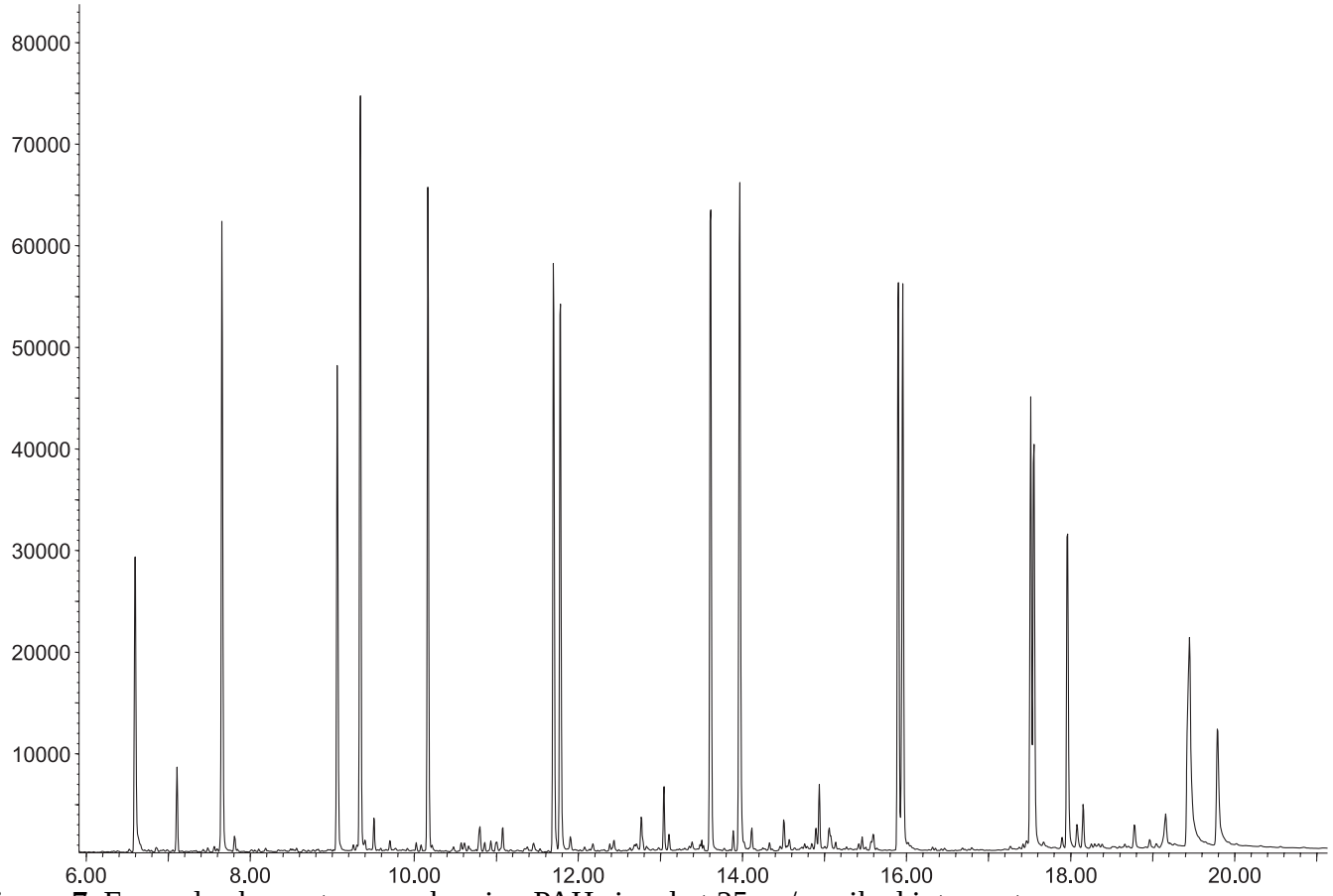


Figure 7. Example chromatogram showing PAH signal at 25 ng/g spiked into oyster.

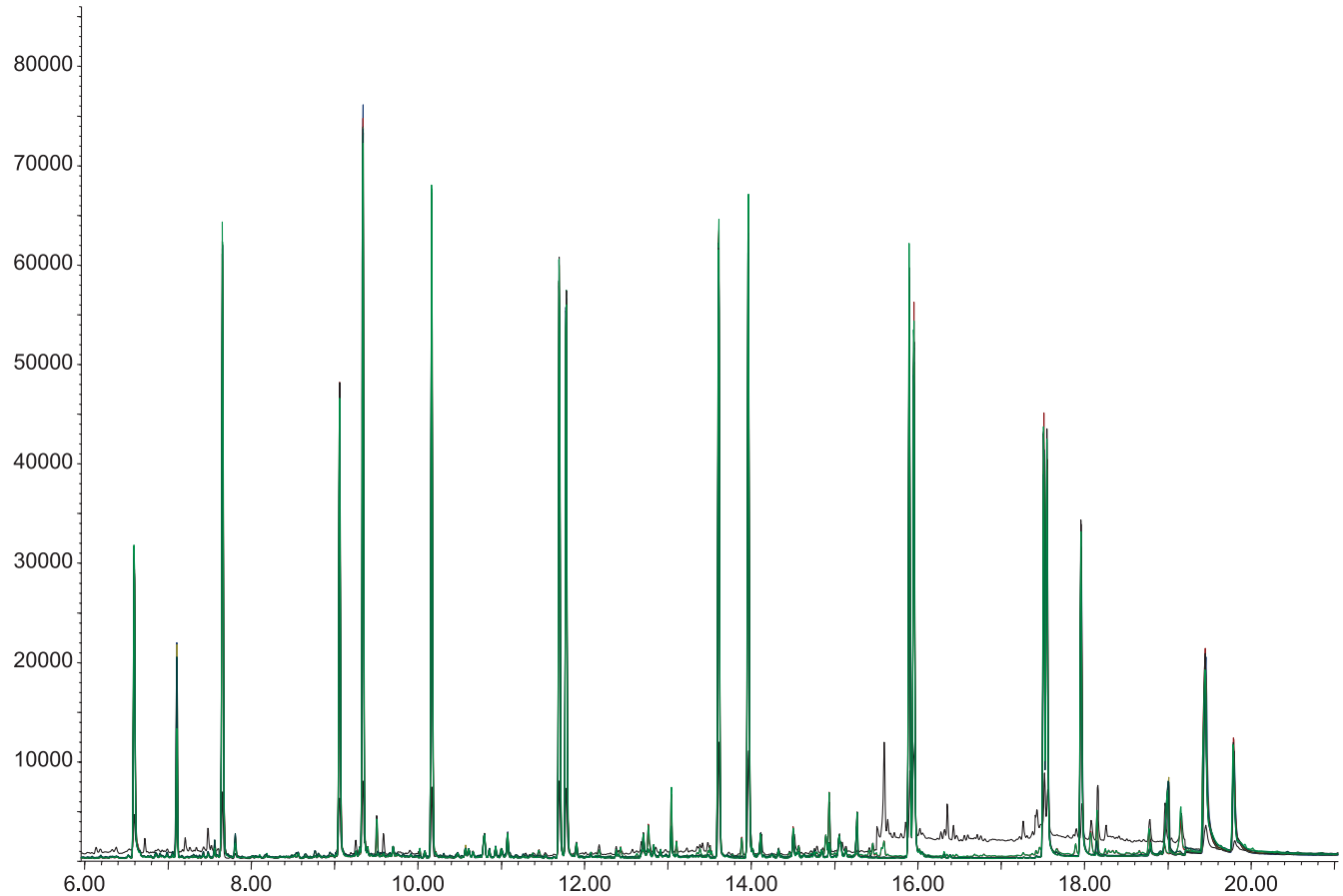
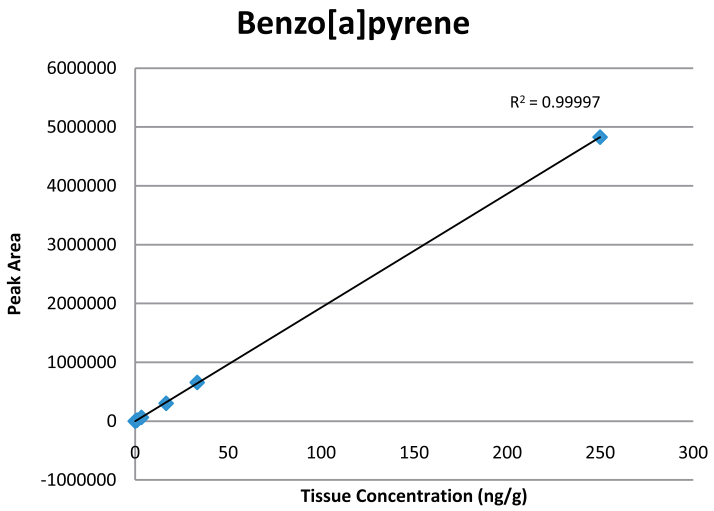


Figure 8. Eight overlaid chromatograms from 18 samples analyzed overnight illustrate retention time stability.



ppb spiked in Tissue Sample	2.5	25.0	25.0
Naphthalene	2.8	29.0	31.1
Fluorene	2.4	29.0	28.5
Phenanthrene	3.4	27.6	27.9
Anthracene	2.9	27.6	27.8
Fluoranthene	2.9	25.2	25.8
Pyrene	2.6	24.4	25.1
Benz[a]anthracene	2.2	23.9	24.4
Chrysene	2.2	25.2	25.0
Benzo[a]pyrene	2.6	25.1	23.1
AVG	2.7	26.3	26.5

Figure 9. Example calibration curve and spike/recovery results for benzo[a]pyrene.

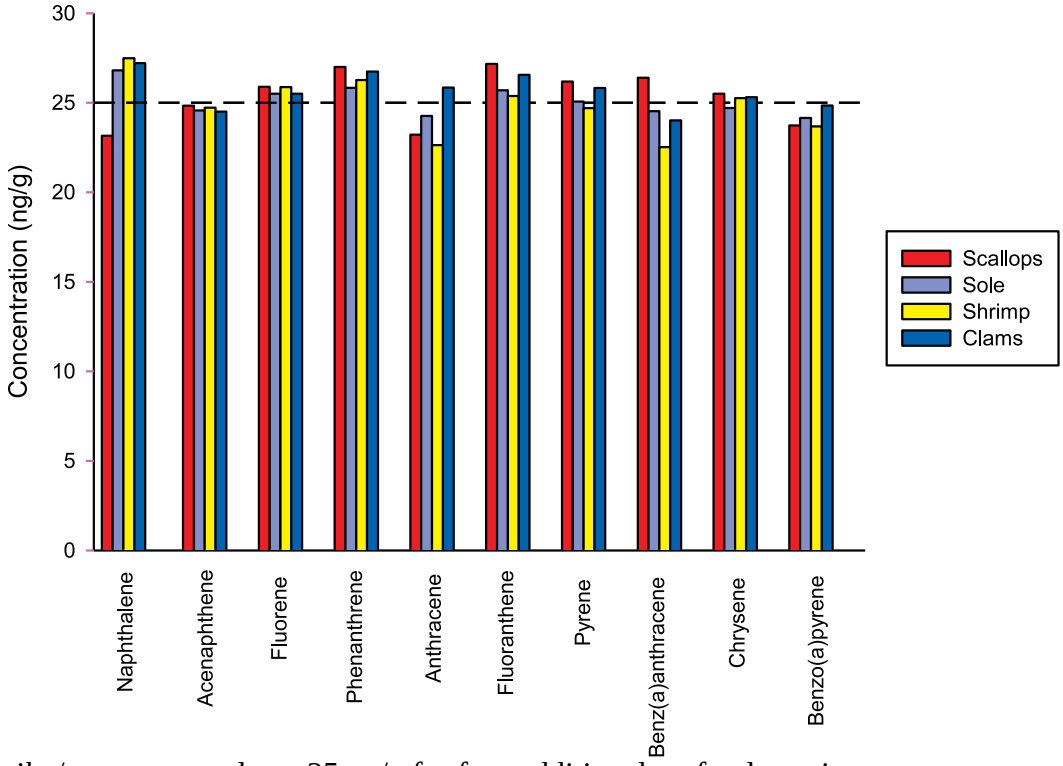


Figure 10. Spike/recovery results at 25 ng/g for four additional seafood matrices.

Analyte	Acceptable range	SRM		
		Certificate of Analysis	QuEChERS SBSE	% RSD
Naphthalene	1.6 - 3.3	2.4	1.1	18.5
Fluorene	0.3 - 0.7	0.49	0.35**	14.7
Phenanthrene	1.7 - 3.5	2.6	1.9	11.1
Anthracene	0.3 - 0.8	0.53	2.4*	15.2
Fluoranthene	11.5 - 23.1	17	19	7.0
Pyrene	12.2 - 24.2	18	18	7.2
Benz[a]anthracene	2.9 - 6.9	4.7	3.5	7.2
Chrysene + Triphenylene	7.4 - 13.8	10.6	8.6	6.8
Benzo[a]pyrene	2.0 - 3.6	2.8	1.7	10.4
Total		59.12	58.05	

* Possible PCB coelution
**n=3 (Only detected using 60 m column)

Figure 11. SRM analysis results.

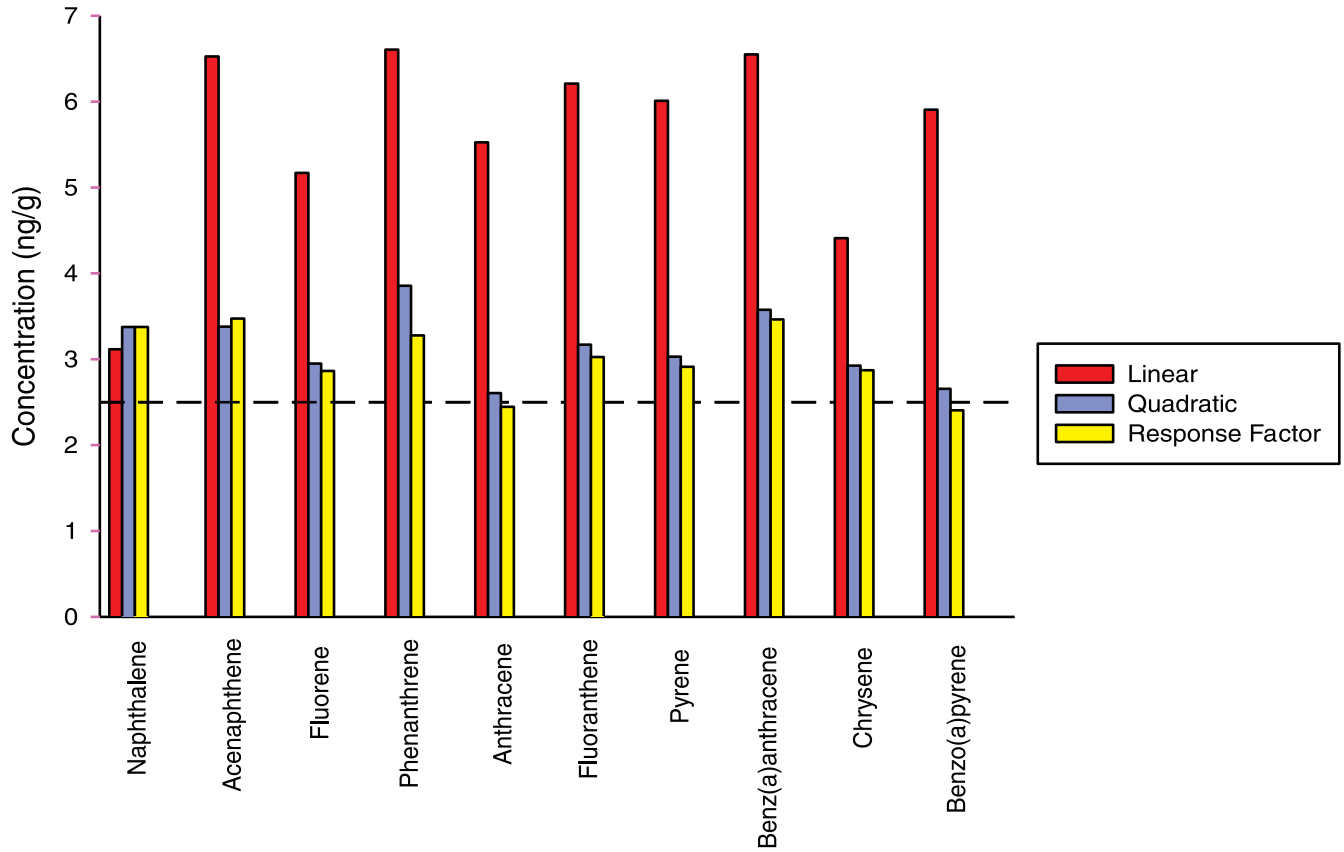


Figure 12. Spike/recovery results at 2.5 ng/g with 3 different calibrations.

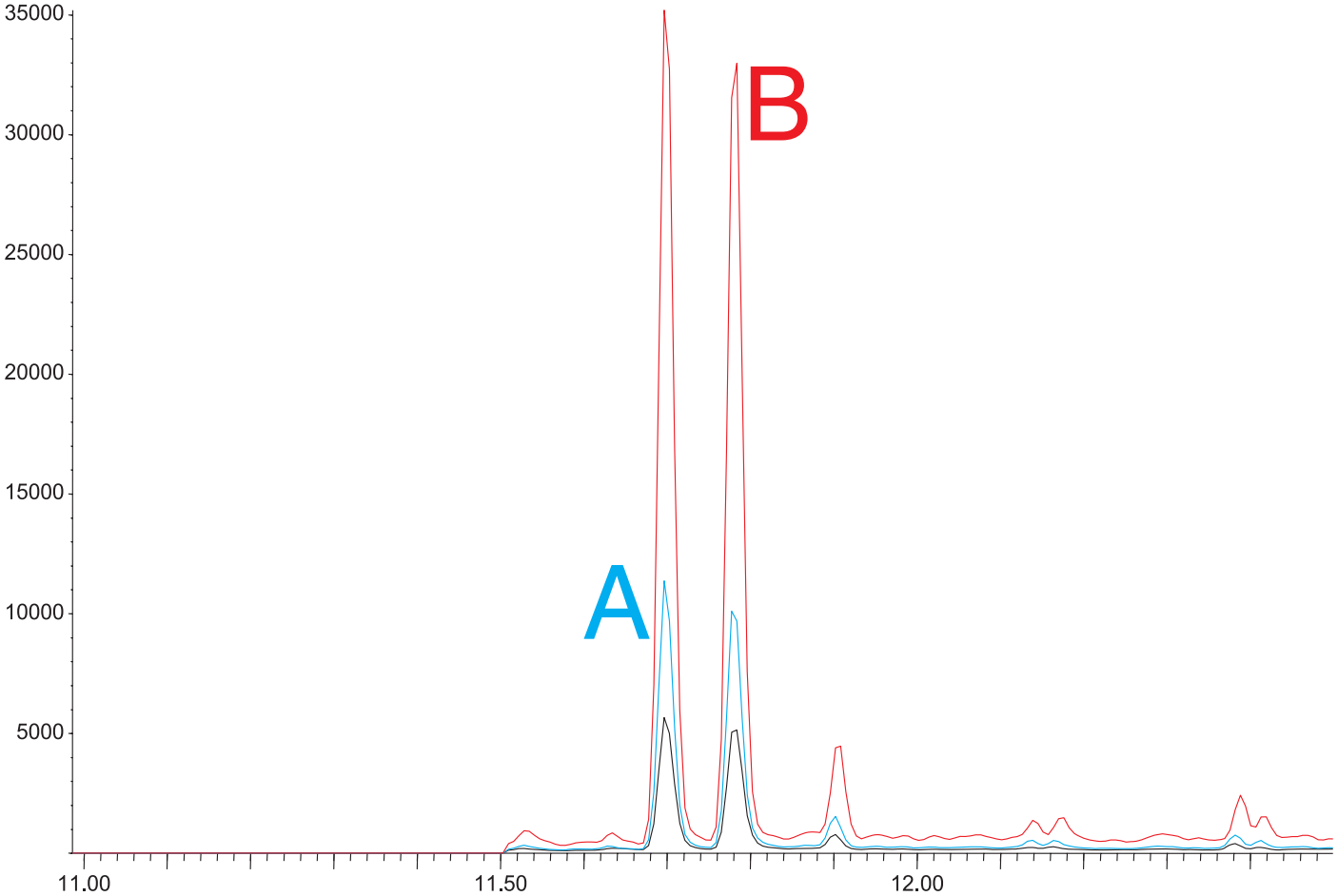


Figure 13. Two approaches to further lower detection limits include desorbing 2 Twisters (A) and using splitless transfer instead of 10:1 split (B).

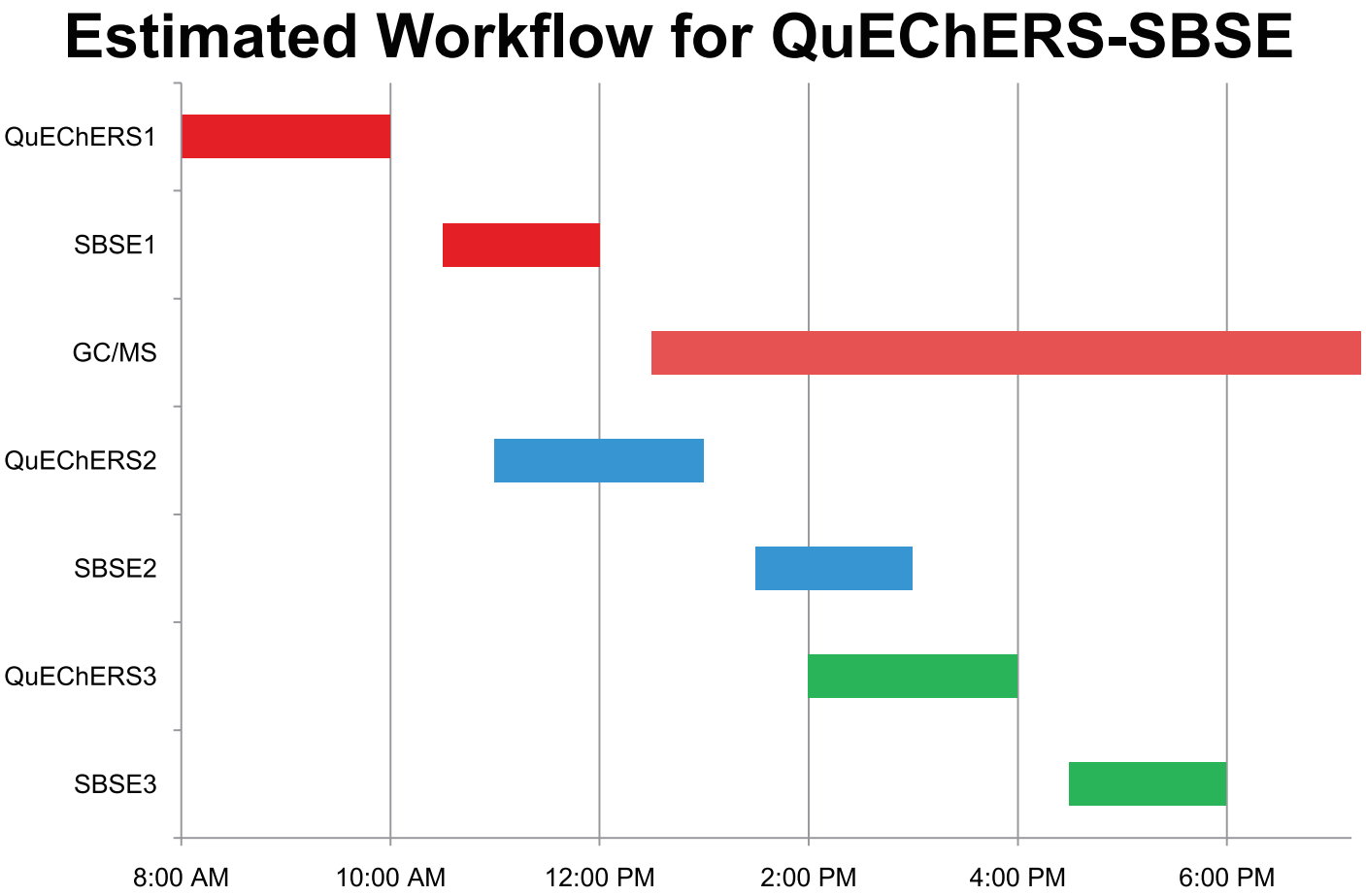


Figure 14. Estimated workflow shows that it is possible to process at least 40 samples per workday.

Conclusions

- The method shows good linearity over the range 1-250 ng/g.
- The analysis of the standard reference material shows good agreement with the actual values.
- Validation data from two labs shows the method can be easily transferred.
- The combination of QuEChERS extraction with SBSE for concentration and matrix management allows accurate single digit ppb detection of the PAHs in marine tissues with the potential for even lower detection limits.
- SBSE provides excellent sample cleanup and method stability.
- The sample throughput is estimated at 40-60 samples per analyst per instrument per day.

Acknowledgments

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