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QuEChERS Sample Preparation for the Determination of Polycyclic Hydrocarbons (PAH) in Shrimp Using LC with Fluorescence Detection

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Abstract

Major oil spills in the world's oceans have brought attention to the problem of PAH contamination, and to the challenges related to PAH analysis in food and environmental samples. QuEChERS sample preparation, followed by LC with fluorescence detection, provides fast screening analysis of PAHs.

Benefits

- Extraction of toxic compounds from shrimp using QuEChERS methodology in under 10 minutes.
- Rapid PAH screening analysis by UPLC in under 4 minutes.
- Low ppb detection limits suitable for screening shrimp from petroleum-contaminated waters.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are toxic compounds commonly found in nature, and are constituents of coal and petroleum. The US EPA has classified seven PAHs as probable human carcinogens. The US FDA has set the limit of concern (LOC) for benzo[a]pyrene, one of the most widely occurring and potent PAHs, at 35 ppb in shellfish. Major oil spills in the world's oceans have brought attention to the problem of PAH contamination, and to the challenges related to PAH analysis in food and environmental samples. This application describes a QuEChERS-based sample preparation protocol for the determination of PAH in shrimp. QuEChERS methodology is quicker, easier, cheaper, safer than, and as rugged as prior methods of sample preparation. The methodology is now commonly used worldwide for sample preparation of fruits and vegetables prior to GC-MS or LC-MS for the determination of pesticide residues. This application note demonstrates that the QuEChERS extraction and cleanup technology can be applied to other types of analytes, and other types of sample matrices as an alternative to more cumbersome methods of sample preparation.

Experimental

Sample description (QuEChERS):

Place 15 g homogenized shrimp into a 50-mL centrifuge tube (p/n 186006814). Add 15 mL acetonitrile (ACN), and shake the tube vigorously for 1 min. Add contents of DisQuE pouch salts for AOAC QuEChERS (p/n 186006812), and shake vigorously for 1 min. Centrifuge for 3 min at 4000 rpm, and take a suitable portion for LC-FLR analysis.

LC conditions

System:	ACQUITY UPLC H-Class with Large Volume Flow Cell (LVFC)
Column:	PAH 4.6 x 50 mm, 3 µm
Column temp.:	35.0 °C
Mobile phase A:	Water
Mobile phase B:	Methanol
Mobile phase C:	Acetonitrile
Flow rate:	2.0 mL/minute
Gradient:	30% A, 70% B initial, linear gradient to 70% B, 30% C at 2.25 min, to 100% C at 3.5 min, back to 30% A, 70% B at 3.6 min, and re-equilibrate.
Injection volume:	10 µL
Detection:	Fluorescence (FLR) using programmed wavelength changes
Vials:	LCGC Certified Vials (p/n 186000307C)

Fluorescence Program:

Time	Excitation (nm)	Emission (nm)
0.00	276	331
1.00	295	315
1.33	248	380
1.62	246	488
1.97	275	380
2.40	300	422
2.70	364	408
2.89	298	410
3.17	305	500

Results and Discussion

Figure 1 shows a LC-FLR chromatogram obtained from the analysis of a shrimp sample spiked at 20 ng/g (ppb) of PAH compounds. PAH recovery was measured at 20 and 200 ppb (n = 5 for each level). Recovery data, shown in Table 1, generally ranged from 83% to 99%. Recovery was calculated by comparing the peak area for samples spiked prior to QuEChERS extraction (pre-spiked samples) with the peak area for samples spiked after QuEChERS extraction (post-spiked samples).

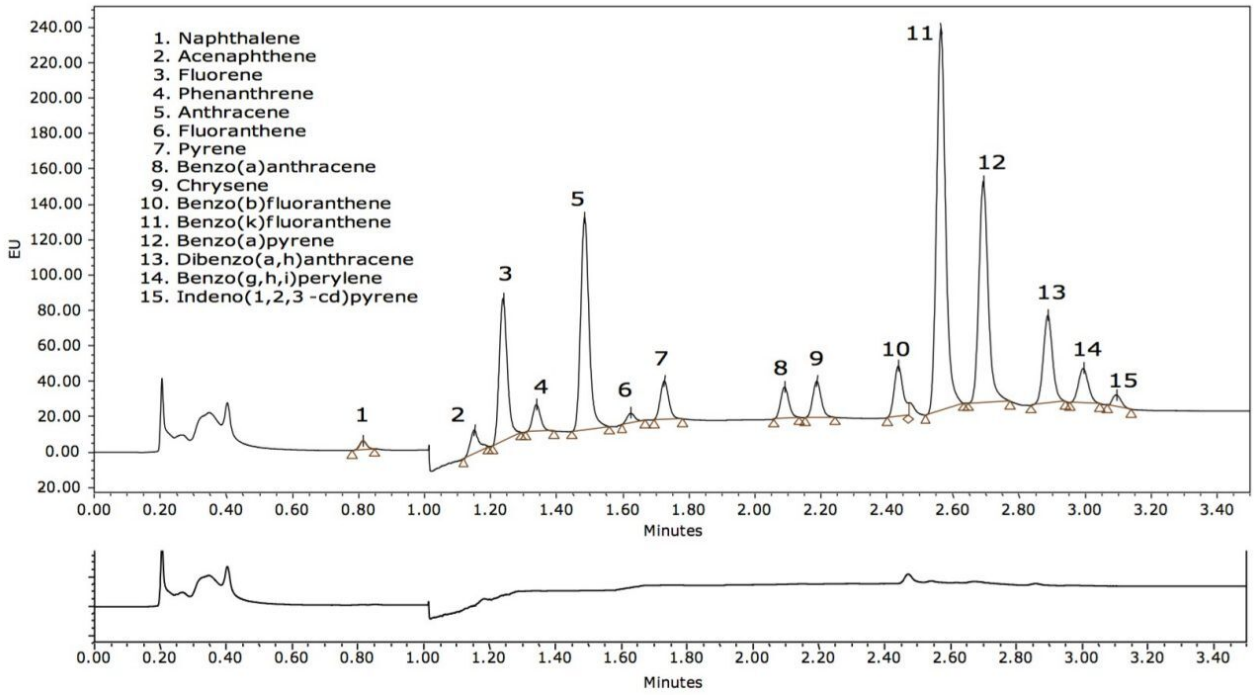

Figure 1. LC-FLR chromatogram obtained from a shrimp sample spiked with 20 ppb PAH compounds. The bottom chromatogram is an unspiked shrimp blank.

PAH Compound	% Recovery (%RSD)	
	20 ppb spike	200 ppb spike
Naphthalene	73 (21)	86 (3.5)
Acenaphthene	83 (9.1)	91 (0.8)
Fluorene	87 (5.4)	93 (0.9)
Phenanthrene	93 (4.0)	94 (1.5)
Anthracene	93 (4.1)	94 (1.4)
Fluoranthene	94 (3.9)	94 (1.9)
Pyrene	94 (4.2)	92 (2.1)
Benzoanthracene	94 (5.2)	90 (2.2)
Chrysene	95 (5.1)	97 (2.3)
Benzo(b)fluoranthene	88 (3.1)	88 (2.4)
Benzo(b) fluoranthene	98 (5.1)	87 (1.7)
Benzo(a) pyrene	88 (6.0)	83 (2.6)
Dibenzo(a,h)anthracene	99 (6.1)	84 (3.6)
Benzo(g,h,i)perylene	96 (6.3)	73 (2.8)
Indeno(1,2,3 -cd)pyrene	98 (8.6)	98 (14.0)

Table 1. Recovery from DisQuE extraction of 20 ppb and 200 ppb spiked shrimp samples.

Recently, two application notes were published that demonstrated the utility of QuEChERS sample preparation applied to the determination of PAH residues in seafood, using LC with FLR detection (LC-FLR),[1] and tandem GC-MS after a further sample cleanup of the QuEChERS extract.[2] The results showed very good accuracy and precision. In this follow-up application, the DisQuE pouch format was used to prepare the homogenized shrimp samples to simplify the addition of the QuEChERS salts. Results were equivalent to those results reported in reference 1.

If GC-MS analysis is preferred, the cleanup procedure presented in reference 2 is recommended.

Conclusion

- The DisQuE pouch product was shown to be effective for the determination of 15 PAHs in shrimp, with consistent recoveries ranging from 80% to 100%.
- QuEChERS sample preparation, followed by LC with fluorescence detection, provides fast screening

analysis of PAH. A batch of ten samples can be processed and analyzed in less than three hours.

- Low ppb detection limits are obtained for most PAHs.

References

1. Benvenuti ME, Burgess JA. Ensuring Seafood Safety with Rapid Screening for Polyaromatic Hydrocarbons Using LC/Fluorescence. Waters Application Note 720003891en. 2011 March.
2. Young MS, Benvenuti ME, Burgess JA, Fountain KJ. Determination of PAH in Seafood: Optimized Sample Preparation Procedures for LC/Fluorescence Screening and GC/MS/MS Confirmation. Waters Application Note 720004126en. 2011 Sept.

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