

SECTION F

QUALITY ASSURANCE

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Quality Assurance for Analyses for Fluorescent Aromatic Compounds (FACs)

Quality assurance procedures for bile analyses included use of naphthalene (NPH) and phenanthrene (PHN) calibration standards, a “bile pool” control material, blank analyses, and replicate analyses to evaluate the performance of the high performance liquid chromatography fluorescence (HPLC/UV-F) method.

Quality Assurance for Analyses for Aromatic Compounds

Quality control (QC) measures included analyses of method blanks, spiked blanks, matrix spikes, and National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1974. Where possible, primary standard solutions were purchased. The recoveries of the following surrogates are reported as QC information: Naphthalene-d8, acenaphthene-d10, and benzo[*a*]pyrene-d12. Analyte concentrations are reported on the basis of the internal standards (“surrogates”) added at the beginning of the sample extraction. The HPLC internal standard (used to determine the fraction of total sample extract used in the analysis for aromatic hydrocarbons) was phenanthrene-d10. Hexamethylbenzene was used as the gas chromatography (GC) internal standard to calculate the recoveries of the surrogates. The criterion for the recovery of each surrogate standard was greater than 50%, but less than 130%, and the relative standard deviations (RSDs) for surrogate recoveries in a set of samples was less than 25%. When the recovery of any surrogate was outside these limits, corrective action was taken, including instrument repair, inlet cleaning, column replacement, and/or reanalysis of the samples.

The GC calibration standards generally included all surrogates and analytes of interest, except for some classes of alkylated aromatic hydrocarbons and dibenzothiophenes. In these cases, the response factor for the corresponding

unsubstituted compound was used to calculate the analyte concentrations. Graduated concentrations of GC-calibration standards were used for determining multilevel response factors. A GC-calibration standard was analyzed after every six samples to demonstrate the stability of the calibration. The GC/MS was considered “under control” when the response for each analyte or surrogate in a GC-calibration standard was reproducible within $\pm 10\%$ from analysis to analysis.

Target detection limits were ≤ 0.5 ng/g (wet weight basis). The instrument sensitivity, the sample weights for the associated sample set, the low-level instrument calibration standards, and the standard reference material were used to determine the detection limits.

One method blank was analyzed with each set of approximately 10 samples. The aromatic hydrocarbons (except NPH, which was present in the solvents at trace concentrations) and dibenzothiophenes in the blanks should not be present at the limit of detection. A matrix spike (containing 50-80 ng/g of each analyte) or a spiked blank (containing 40-70 ng/g of each analyte) was analyzed with each set of approximately 10 samples during the first year (about 1/3) of the study. During the latter part of the study, a sample of NIST Standard Reference Material 1974 was analyzed with each set of 10 samples.

The percent recovery of the surrogate standards provides a measure of losses incurred for each sample during the extraction and cleanup steps. Ideally, recoveries would be near 100%, but realistically, recoveries of approximately 90% are considered within quality control guidelines because the procedures involve so many sample handling steps. The mean percent recovery and standard deviation of the surrogate standards (deuterated naphthalene, deuterated acenaphthene, and deuterated benzo[a]pyrene) were $92\% \pm 15\%$.

Method detection limits are usually defined by a combination of factors including instrument signal-to-noise ratio, the lowest level of multilevel standard that can be reliably measured, the original sample weight, and the final volume of the sample extract. Based on the results for field blanks (Table F1), method blanks analyzed with each set of samples (Table F2), and results for the analyses of the NIST SRM 1974 analyzed with each set of samples after the first year (Table F3), our detection limits ranged from about 0.5 ng/g to about 1 ng/g. One of the

certified values in the NIST SRM is 0.35 ng/g for dibenz[a,h]anthracene (DBA). Our results for DBA were 0.3 ng/g \pm 0.4 ng/g.

Naphthalene was present in the solvents at trace concentrations and resulted in concentrations in method blanks and in samples of about 1 ng/g (Table F2). Generally, the other analytes were not found in the method blanks at the stated limit of detection.

In the first year of the study, analytes were added to a method blank (also called a spiked blank) or to a tissue sample (also called a matrix spike) and then analyzed as a sample with each set of samples. The results were used to show that the analytes would be found by this method and to show losses due to sample handling but would not provide any information on extraction efficiency. The recoveries for the analytes in spiked blanks (Table F4) and in matrix spikes (Table F5) ranged from 75% to 130%. As soon as SRM 1974 tissue material was available, a sample of it was analyzed with every set of samples in place of a spiked blank or a matrix spike. The SRM was far more useful than the matrix spike for quality control because it was a homogeneous material, 11 analyte values were certified or noted, and its use afforded a way to monitor extraction efficiency as well as losses due to sample handling. Also, the SRM tissue contained all of the analytes we were monitoring in the subsistence samples, thus enabling us to monitor quality control for all of our analytes.

QA TABLES

Table F-1. Detection limits and standard deviations, ng/g (ppb) wet weight, of aromatic compounds (ACs) in field blanks (n=10). The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

ACs	Mean	Standard deviation
C1-naphthalenes	< 0.5	0.4
C2-naphthalenes	< 0.6	1
C3-naphthalenes	< 0.4	0.6
C4-naphthalenes	< 0.2	0.2
acenaphthylene	< 0.2	0.2
acenaphthene	< 0.3	0.2
fluorene	< 0.2	0.2
C1-fluorenes	< 0.2	0.1
C2-fluorenes	< 0.2	0.1
C3-fluorenes	< 0.2	0.2
phenanthrene	< 0.4	0.2
C1-phenanthrenes	< 0.3	0.3
C2-phenanthrenes	< 0.2	0.2
C3-phenanthrenes	< 0.2	0.2
C4-phenanthrenes	< 0.1	0.1
dibenzothiophene	< 0.2	0.3
C1-dibenzothiophenes	< 0.2	0.1
C2-dibenzothiophenes	< 0.1	0.1
C3-dibenzothiophenes	< 0.1	0.1
fluoranthene	< 0.2	0.2
pyrene	< 0.2	0.2
C1-fluoranthenes/pyrenes	< 0.09	0.06
benz[a]anthracene (BAA)	< 0.2	0.2
chrysene	< 0.1	0.1
C1-chrysenes/BAAs	< 0.08	0.03
C2-chrysenes/BAAs	< 0.08	0.03
C3-chrysenes/BAAs	< 0.1	0.1
C4-chrysenes/BAAs	< 0.08	0.04
benzo[b]fluoranthene	< 0.1	0.09
benzo[k]fluoranthene	< 0.1	0.07
benzo[a]pyrene	< 0.1	0.07
indeno[1,2,3-cd]pyrene	< 0.1	0.08
dibenz[a,h]anthracene	< 0.2	0.1
benzo[ghi]perylene	< 0.1	0.1

Table F-2. Detection limits and standard deviations, ng/g (ppb) wet weight, of aromatic compounds (ACs) in method blanks (n=165). The "less than" symbol (<) indicates that the analyte was not detected in concentrations above the stated value.

ACs	Mean	Standard deviation
C1-naphthalenes	< 0.8	0.9
C2-naphthalenes	< 0.4	0.7
C3-naphthalenes	< 0.3	0.3
C4-naphthalenes	< 0.2	0.2
acenaphthylene	< 0.3	0.8
acenaphthene	< 0.3	0.3
fluorene	< 0.2	0.2
C1-fluorenes	< 0.2	0.2
C2-fluorenes	< 0.2	0.2
C3-fluorenes	< 0.3	0.3
phenanthrene	< 0.4	0.7
C1-phenanthrenes	< 0.1	0.2
C2-phenanthrenes	< 0.1	0.2
C3-phenanthrenes	< 0.1	0.1
C4-phenanthrenes	< 0.1	0.1
dibenzothiophene	< 0.1	0.1
C1-dibenzothiophenes	< 0.2	0.1
C2-dibenzothiophenes	< 0.2	0.1
C3-dibenzothiophenes	< 0.2	0.1
fluoranthene	< 0.2	0.3
pyrene	< 0.2	0.2
C1-fluoranthenes/pyrenes	< 0.1	0.1
benz[a]anthracene (BAA)	< 0.2	0.4
chrysene	< 0.2	0.4
C1-chrysenes/BAAs	< 0.2	0.4
C2-chrysenes/BAAs	< 0.2	0.4
C3-chrysenes/BAAs	< 0.2	0.4
C4-chrysenes/BAAs	< 0.2	0.4
benzo[b]fluoranthene	< 0.2	0.3
benzo[k]fluoranthene	< 0.1	0.2
benzo[a]pyrene	< 0.2	0.3
indeno[1,2,3-cd]pyrene	< 0.2	0.3
dibenz[a,h]anthracene	< 0.2	0.3
benzo[ghi]perylene	< 0.2	0.2

Table F-3. Mean concentrations, ng/g (ppb) wet weight, of aromatic compounds (ACs) determined in NIST Standard Reference Material 1974, standard deviation (n=121), and certified values.

ACs	Mean	σ	Certified concentrations	
			Mean	σ
naphthalene	4	1		
C1-naphthalenes	3	2	3.2*	0.5
C2-naphthalenes	4	2		
C3-naphthalenes	8	3		
C4-naphthalenes	24	9		
acenaphthylene	0.4	0.6		
acenaphthene	0.6	0.6		
fluorene	1	0.6	1.5*	0.2
C1-fluorenes	4	1		
C2-fluorenes	20	8		
C3-fluorenes	23	15		
phenanthrene	6	2	5.6	1.4
C1-phenanthrenes	11	4		
C2-phenanthrenes	44	15		
C3-phenanthrenes	49	19		
C4-phenanthrenes	11	8		
dibenzothiophene	0.9	0.4		
C1-dibenzothiophenes	5	2		
C2-dibenzothiophenes	29	10		
C3-dibenzothiophenes	34	13		
fluoranthene	46	8	33.6	5.8
pyrene	44	7	34.1	3.7
C1-fluoranthenes/pyrenes	25	7		
benz[a]anthracene (BAA)	5	1	4.6*	0.4
chrysene	18	3		
C1-chrysenes/BAAAs	7	2		
C2-chrysenes/BAAAs	3	2		
C3-chrysenes/BAAAs	0.6	0.9		
C4-chrysenes/BAAAs	0.0	0.4		
benzo[b]fluoranthene	7	2	6.5	1.2
benzo[k]fluoranthene	4	1		
benzo[a]pyrene	2	1	2.29	0.47
indeno[1,2,3-cd]pyrene	2	1.0	1.8	0.3
dibenz[a,h]anthracene	0.3	0.4	0.35*	0.01
benzo[ghi]perylene	2	1.0	2.47	0.28
sample weight, grams:	4	1.0		

* Noncertified concentrations reported by NIST.

Table F-4. Spiked blanks. Mean percent recoveries and relative standard deviations (RSD) of aromatic compounds (ACs) in spiked blanks (n=6).

ACs	Mean %	Relative standard deviation %	Amount spiked ng/g
naphthalene	100	4	65
acenaphthylene	95	7	63
acenaphthene	98	2	69
fluorene	98	4	66
phenanthrene	100	6	68
dibenzothiophene	110	7	120
benz[a]anthracene	120	18	58
chrysene	110	9	68
benzo[b]fluoranthene	110	16	66
benzo[k] fluoranthene	110	12	65
benzo[a]pyrene	96	4	59
indeno[1,2,3-cd]pyrene	110	15	54
dibenz[a,h]anthracene	120	19	43
benzo[ghi]perylene	100	13	56

Table F-5. Matrix spikes. Mean percent recoveries and relative standard deviations (RSD) of aromatic compounds in matrix spikes (n=39).

ACs	Mean %	Relative standard deviation %	Amount spiked ng/g
naphthalene	110	25	65
acenaphthylene	93	16	70
acenaphthene	92	13	63
fluorene	95	21	69
phenanthrene	110	13	68
dibenzothiophene	120	23	120
fluoranthene	130	30	
pyrene	120	30	
benz[a]anthracene	94	25	58
chrysene	94	24	68
benzo[b]fluoranthene	86	20	66
benzo[k]fluoranthene	82	20	65
benzo[a]pyrene	87	17	59
indeno[1,2,3-cd]pyrene	77	26	54
dibenz[a,h]anthracene	76	33	43
benzo[ghi]perylene	75	24	56