Demonstration of New Matrix Spike Procedure for 1613b Method

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Introduction

Method 1613b[1] uses the isotope dilution technique to correct for any loss of analyte through the process of sample extraction, cleanup, and GC/MS analysis. Using the isotope dilution technique for polychlorinated dibenzo-dioxins and polychlorinated dibenzo-furans (CDD/CDFs) analysis can be an accurate way to adjust for analyte loss as the ^{13}C analyte behaves almost identically to the native CDD/CDF. A major assumption, however, with this technique is that one can distribute the ^{13}C CDD/CDFs as the native CDD/CDFs are distributed in the matrix. For aqueous samples, method 1613b distributes the ^{13}C CDD/CDF by adding them in an acetone solution to the sample and allowing it to equilibrate for an hour or two. The assumption here is that the CDD/CDFs will bind with any particles in the sample. In the instance of the matrix spike for 1613b, CDD/CDFs have no particles to bind to because reagent water is used as the matrix. Therefore, the procedure is testing the analyst's ability to extract CDD/CDFs from a very atypical type of sample.

The purpose of this study was to test a new matrix spike procedure that would more accurately reflect "real world" samples and compare the precision and recovery results to 1613b acceptance criteria. The new procedure involved spiking CDD/CDFs directly onto solid particles and then adding the particles to reagent water. The reagent water containing the solid particles was filtered and then the particles and filter paper are spiked with ^{13}C CDD/CDFs. This new matrix spike procedure better represents the matrix found in actual samples and it was the hypothesis that this in turn would better demonstrate and more accurately reflect the capability of the 1613b method.

The acceptance criteria for the initial precision and recovery (IPR) of the individual 2,3,7,8-substituted CDD/CDFs are shown in Table I & II. In Table I, the average percent relative standard deviation (RSD) and average allowable range for recoveries of native polychlorinated dibenzo-dioxins and polychlorinated dibenzo-furans (CDD/CDFs) is 18 and 50, respectively. Basically, these criteria allow for significant variability in the measurement of the native that had been spiked into the Reagent Water matrix. In Table II the average percent RSD and allowable range of recoveries for ^{13}C-labeled CDD/CDFs is 39 and 120, respectively. This allowed variability in the ^{13}C-labeled CDD/CDFs would significantly affect the accuracy of analyte measurements that are made on real-world samples which contain native analytes that are present on particles in water samples. The combination of the allowed variabilities of native and ^{13}C-labeled analytes could result in a significant bias in the measurement of CDD/CDFs in water samples. For example, a

positive bias could arise due to low recoveries of ^{13}C-labeled internal standards because they were not distributed properly in the sample and relatively higher recoveries of native analytes present on particles.

Methods and Materials

Matrix Spiked Sand

1 gram of sand (Sand, standard Ottawa, EM Science, CAS# 14808-60-7; pre-extracted in benzene) was added into a 1-dram vial. To the vial, 0.5mL of matrix spike stock solution (See Table III for list of analytes and concentrations) was spiked onto the sand and then completely evaporated under a stream of nitrogen. After evaporation, the sides of the vial were rinsed down with hexane and the hexane was allowed to evaporate naturally overnight to evenly distribute the native CDD/CDFs.

2,3,7,8-TCDD Matrix Spiked Sand

1 gram of sand (Sand, standard Ottawa, EM Science, CAS# 14808-60-7; pre-extracted in benzene) was added into a 1-dram vial. To the vial, 4µL of 2,3,7,8-tetrachlorodibenzo-*p*-dixion (2,3,7,8-TCDD) matrix spike stock solution (3.9pg) was spiked onto the sand. The sides of the vial were rinsed down with hexane and the hexane was allowed to evaporate naturally overnight to evenly distribute the native CDD/CDFs.

Matrix Spike Sample Preparation

The Matrix Spiked Sand was added into a bottle containing approximately 800g of water. The bottle was then occasionally shaken by hand over a 60-minute period. After 60 minutes, the water solution was vacuum filtered through a Buchner funnel containing 1.2 µm Whatman GF/C filter paper into an Erlenmeyer flask. The sample bottle was rinsed with water until the majority of particles were removed. The filter paper was then removed from the Buchner funnel, spiked with 10 µL of internal standard (100, 40, 40, 40, 100ppb), and Soxhlet-Dean-Stark extracted overnight. The sample bottle, Buchner funnel and Erlenmeyer flask were washed with 40ml of acetone and 40ml of a 20% benzene / hexane solution. The two solutions were added to the filtrate and the combined solution was stirred overnight to extract any dissolved CDD/CDFs.

The SDS extract and 20% benzene/hexane liquid extract were combined and processed through three classical liquid chromatography columns (CLCC). The details of the sample clean-up procedure through the three CLCC are given in Reference 2. The final extract was then analyzed by High Resolution Gas Chromatography/ High Resolution Mass Spectrometry (HRGC/HRMS)

HRGC/HRMS

The measurements were performed with HRGC/HRMS system based on ThermoQuest Series 2000 Gas Chromatograph and Finnigan MAT-95S magnetic sector instrument. The mass spectrometer was equipped with a standard electron ionization source operating in positive ionization mode. Typical ionization conditions were electron energy of 40eV, ion source temperature of 240 °C, and acceleration voltage of 4767V. The mass spectrometer was operated at >9000 resolution (10% valley) using the linear voltage scanning procedure in the centroid mode acquisition. All samples were introduced into the GC inlet by LEAP CTC A200SE autosampler. The GC column used was standard non-polar (5% phenyl) methyl polysiloxane phase column.

Results and Discussion

The results for the initial precision and recovery experiment are shown in Table III for the native CDD/CDFs. When compared to acceptance criteria for 1613b shown in Table I, there are two striking differences. First, the average recovery for each 2,3,7,8-substituted CDD/CDFs isomer is close to 100%. Actually, the overall average recovery for the 2,3,7,8-substituted CDD/CDFs was 99% with a RSD of 6%. Compare this to Table I where the average allowed range for recoveries is 50%. The second noteworthy difference is the observed precision for this new matrix spike method. In all cases, when using the matrix spiked sand, the precision was a factor of 2 to 13 times greater than the acceptance criteria for 1613b for all the 2,3,7,8-substituted CDD/CDFs.

The internal standard results from the initial precision and recovery experiment are shown in Table IV. Again, there are two noteworthy differences. As with the native CDD/CDFs, the average recovery for all internal standards using the matrix spiked sand was 90% with a RSD of 4%. Compare this to 1613b acceptance criteria, where the average minimum and maximum is 28 and 148, respectively. For the precision comparison, the matrix spike sand's relative standard deviations were a factor of 7 to 23 times less than the allowed relative standard deviations in 1613b.

This noted overall improvement in precision and recovery ultimately relates to a better understanding of the method's capability. For example, using 1613b criteria, an analyst during an experiment could lose 88% of the native 2,3,4,7,8-pentachlorodibenzo-*p*-furan (2,3,4,7,8-PeCDF) and still pass.

Percent loss = (1 - (recovery of native) x (recovery of internal standard)) x 100
= (1 - 0.72 *0 .16) * 100
= 88

Compare this to a 22% loss of 2,3,4,7,8-PeCDF calculated using the lowest native and internal standard recoveries in the new matrix spiked sand experiment for 2,3,4,7,8-PeCDF and using the noted sample extraction and cleanup procedures.

Percent loss = (1 - (recovery of internal standard) x (recovery of native)) x 100
= (1 - 0 .90 *0 .87) * 100
= 22

Considering the International Toxicity Equivalency Factor (I-TEF)[3] for 2,3,4,7,8-PeCDF is 0.5, any difference in the amount recovered for 2,3,4,7,8-PeCDF could have a significant impact on the Total Toxic Equivalency (TEQ) calculation.

$$\text{Total Toxic Equivalency} = \sum_{n=1}^{k} C_n * ITEF_n$$

C_n is the concentration of the individual I-TEF.

The most likely reason for the difference between an acetone matrix spike and a sand matrix spike is that the CDD/CDFs in the acetone spike are forced to dissolve in water. This causes an inherent problem due to CDD/CDFs being very water insoluble. Hence, when spiked into water, the

CDD/CDFs will likely adhere to other surfaces such as the glass of the container. This is not an accurate representation of an "actual sample" which most likely has small amounts of solids where the CDD/CDFs will be located.

Table V shows the on-going precision and recovery results for 2,3,7,8-TCDD only matrix spike. The results in Table V are within 1613b acceptance criterion of 87%-124% recovery when only 2,3,7,8-TCDD is tested. The purpose of this test was to demonstrate the applicability of this technique to a low level spike. Despite the level tested of 2,3,7,8-TCDD being approximately 50 times lower than the test level in 1613b, it still passed the recovery acceptance criterion.

Conclusion

The concept of using a native CDD/CDF matrix spike quality assurance element in analytical methods is to ascertain the ability of the procedure to accurately determine the analytes that have been physically added to a sample. The standard EPA approach to preparing this spiked water sample uses an acetone solution of CDD/CDFs to fortify a Reagent Water matrix. This is perhaps the most unnatural mechanism for introducing CDD/CDFs to water. In most chemical or environmental processes the CDD/CDFs are adsorbed on particles before they are emitted into a water matrix. The proposed spiking mechanism that has been presented in this report is a much better simulation of a real-world situation. Using CDD/CDFs spiked onto sand as a substitute to a CDD/CDFs acetone solution for matrix spiking meets all the IPR data requirements of 1613b and has the potential to be used for lower level spikes of CDD/CDFs. In fact, the matrix-spiked sand has shown the ability to greatly reduce the variability and enhance the recovery when compared to an acetone solution.

References

1. Method 1613 Revision B, Tetra – Through Octa – Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, EPA, October 1994, Washington, D.C.
2. Method 4: Determination of specific halogenated dibenzo-*p*-dioxin and dibenzofuran isomers in environmental and biological matrices by gas chromatography-mass spectrometry, Environmental Carcinogens Methods of Analysis and Exposure Measurement, IARC Scientific Publications No. 108, Volume 11, International Agency for Research on Cancer, 1991, Lyon.
3. Interim procedures for estimating risks associated with exposures to mixtures of chlorinated dibenzo-p-dioxins and -dibenzofurans (CDD/CDFs) and 1989 update, EPA, EPA/625/3-89/016, Washington D.C.

TABLE I

1613b Initial Precision and Recovery (IPR) Acceptance Criteria for Performance Tests when all Native CDD/CDFs are Tested

Analyte	Test Conc (pg)	RSD (%)	Min Rec (%)	Max Rec (%)
2,3,7,8-TeCDD	200	28	83	129
1,2,3,7,8-PeCDD	1000	15	76	132
1,2,3,4,7,8-HxCDD	1000	19	78	152
1,2,3,6,7,8-HxCDD	1000	15	84	124
1,2,3,7,8,9-HxCDD	1000	22	74	142
1,2,3,4,6,7,8-HpCDD	1000	15	76	130
OCDD	2000	19	89	127
2,3,7,8-TeCDF	200	20	87	137
1,2,3,7,8-PeCDF	1000	15	86	124
2,3,4,7,8-PeCDF	1000	17	72	150
1,2,3,4,7,8-HxCDF	1000	17	82	118
1,2,3,6,7,8-HxCDF	1000	13	92	120
2,3,4,6,7,8-HxCDF	1000	15	74	148
1,2,3,7,8,9-HxCDF	1000	13	84	122
1,2,3,4,6,7,8-HpCDF	1000	13	90	112
1,2,3,4,7,8,9-HpCDF	1000	16	86	126
OCDF	2000	27	74	146

TABLE II

1613b IPR Acceptance Criteria for Performance Tests when all ^{13}C CDD/CDFs are Tested

Analyte	Test Conc (pg)	% RSD	Min Rec (%)	Max Rec (%)
2,3,7,8-TeCDD	2000	37	28	134
1,2,3,7,8-PeCDD	2000	39	27	184
1,2,3,4,7,8-HxCDD	2000	41	29	147
1,2,3,6,7,8-HxCDD	2000	38	34	122
1,2,3,7,8,9-HxCDD	NR	NR	NR	NR
1,2,3,4,6,7,8-HpCDD	2000	35	34	129
OCDD	4000	48	21	138
2,3,7,8-TeCDF	2000	35	31	113
1,2,3,7,8-PeCDF	2000	34	27	156
2,3,4,7,8-PeCDF	2000	38	16	279
1,2,3,4,7,8-HxCDF	2000	43	27	152
1,2,3,6,7,8-HxCDF	2000	35	30	122
2,3,4,6,7,8-HxCDF	2000	37	29	136
1,2,3,7,8,9-HxCDF	2000	40	24	157
1,2,3,4,6,7,8-HpCDF	2000	41	32	110
1,2,3,4,7,8,9-HpCDF	2000	40	28	141
OCDF	NR	NR	NR	NR

TABLE III
Initial Precision and Recovery Results for Native CDD/CDFs

Analyte	Spike level (pg)	Samp. 1 (%)	Samp. 2 (%)	Samp. 3 (%)	Samp. 4 (%)	Samp. 5 (%)	Ave. Rec.	RSD (%)
2,3,7,8-TeCDD	198	103	94	89	85	94	93	7.2
1,2,3,7,8-PeCDD	984	95	96	92	93	94	94	1.7
1,2,3,4,7,8-HxCDD	840	103	101	96	103	107	102	3.9
1,2,3,6,7,8-HxCDD	1126	106	101	95	89	87	95	8.5
1,2,3,7,8,9-HxCDD	856	114	112	103	109	109	109	3.7
1,2,3,4,6,7,8-HpCDD	1027	104	104	103	97	100	102	3.0
OCDD	1930	105	103	107	105	104	105	1.5
2,3,7,8-TeCDF	205	97	93	91	83	89	91	5.4
1,2,3,7,8-PeCDF	954	103	96	95	96	92	96	4.1
2,3,4,7,8-PeCDF	958	101	100	93	90	97	96	4.9
1,2,3,4,7,8-HxCDF	918	86	93	91	102	97	94	6.7
1,2,3,6,7,8-HxCDF	938	108	108	110	107	99	106	4.1
2,3,4,6,7,8-HxCDF	943	104	106	99	99	100	102	3.0
1,2,3,7,8,9-HxCDF	993	105	100	101	92	99	99	4.7
1,2,3,4,6,7,8-HpCDF	949	110	106	104	104	104	106	2.5
1,2,3,4,7,8,9-HpCDF	988	92	97	102	91	98	96	4.8
OCDF	1910	106	107	105	102	103	105	2.0

TABLE IV
Internal Standard Initial Precision and Recovery Results (^{13}C labeled)

Analyte	Spike Level (pg)	Samp. 1 (%)	Samp. 2 (%)	Samp. 3 (%)	Samp. 4 (%)	Samp. 5 (%)	Ave. Rec.	RSD (%)
2,3,7,8-TeCDD	1000	82	84	86	85	81	84	2.5
1,2,3,7,8-PeCDD	400	87	91	92	90	89	90	2.1
1,2,3,4,7,8-HxCDD	400	84	89	94	93	91	90	4.4
1,2,3,6,7,8-HxCDD	NR	NR	NR	NR	NR	NR	NR	NR
1,2,3,7,8,9-HxCDD	400	92	91	102	94	94	95	4.6
1,2,3,4,6,7,8-HpCDD	400	84	91	91	96	93	91	4.9
OCDD	1000	93	97	91	97	96	95	2.8
2,3,7,8-TeCDF	1000	78	82	82	81	77	80	2.9
1,2,3,7,8-PeCDF	400	81	88	87	83	83	84	3.5
2,3,4,7,8-PeCDF	400	88	91	94	91	87	90	3.1
1,2,3,4,7,8-HxCDF	400	87	90	89	91	91	90	1.9
1,2,3,6,7,8-HxCDF	NR	NR	NR	NR	NR	NR	NR	NR
2,3,4,6,7,8-HxCDF	400	86	88	94	89	90	89	3.3
1,2,3,7,8,9-HxCDF	400	83	92	89	97	90	90	5.6
1,2,3,4,6,7,8-HpCDF	400	82	90	92	89	89	88	4.3
1,2,3,4,7,8,9-HpCDF	400	94	96	95	102	95	96	3.3
OCDF	1000	93	97	102	101	97	98	3.7

NR = Analytes not contained in internal standard spike solution

TABLE V

2,3,7,8-TCDD: Native and ^{13}C Standard Ongoing Precision and Recovery Results

Sample	2378TCDD (pg)	^{13}C-2378TCDD (%)
Spike Level	3.9	N/A
Sample #1	3.8	73
Sample #2	3.6	75
Sample #3	3.7	72
Sample #4	3.8	90
Sample #5	4.4	91
Sample #6	4.2	103
Sample #7	4.4	129
Sample #8	3.5	90
Average	3.9	90
Average Recovery (%)	101	N/A
% RSD	9	21