Diluting the CS-5 Standard for the Analysis of Low Level PCB Samples by Isotope Dilution HRGC/HRMS Using EPA Method 1668A

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Introduction

In recent years there has been a demand for lower detection limits for the analysis of polychlorinated biphenyls (PCBs). EPA Method 1668A[1] has been developed to detect low level contamination of PCBs in water, sediment and tissue samples. Method 1668A uses several techniques to reach low levels of detection. These techniques include the method of isotope dilution and analysis by high resolution mass spectrometry (HRMS). With isotope dilution, $^{13}C_{12}$ labeled internal standards are added to the sample prior to extraction and sample cleanup. Theoretically the loss of native analytes in sample preparation is corrected for by the proportional loss of the internal standards. Analysis by HRMS at ≥10,000 resolution filters out interferences and noise providing high sensitivity and selectivity. Two exact m/z's are monitored for each native and labeled analyte. The ratio between the two masses is utilized as a qualitative identification criterion.

Method 1668A has a standard five point calibration curve (CS-1 to CS-5) with an optional sixth calibration solution (CS-0.2) to lower detection limits even further. The six calibration solutions cover a native PCB concentration range from 0.2 ng/ml to 2000 ng/ml. We have found that the secondary ions of PCB congeners at lower levels of chlorination in the CS-0.2, such as PCB 4 and 15, typically have low signal-to-noise ratios and require that the mass spectrometer be optimized to increase the sensitivity for these analytes. When the sensitivity of the mass spectrometer is increased sufficiently to detect all CS-0.2 ions, the dynamic range of the instrument (ie. the point of detection from noise to the point of peak saturation) typically does not encompass all the analytes in the CS-5 standard. Some, but not all peaks corresponding to CS-5 analytes are saturated. Options available to prevent the saturation of the high standard include turning the detector down to a point where the CS-5 does not saturate, only analyzing the CS-0.2 through the CS-4, or diluting the CS-5 solution. Turning the detector down will decrease the sensitivity of the instrument and decrease the signal of the lower chlorination groups, which is already a problem at the CS-0.2 level. Removing the CS-5 from the calibration curve will decrease the linear range of the calibration significantly because of the large concentration gap between the CS-4 and CS-5 (400 to 2000 ng/ml). A dilution of the CS-5 solution gives the greatest calibration range possible with maximum sensitivity at the low end of the calibration range. Here we justify diluting the CS-5 solution in order to achieve more accurate results in the analysis of low level samples using Method 1668A.

Materials and Methods

Methods followed Method 1668A. Analysis was performed by a HP 5890 series II gas chromatograph interfaced to a VG Autospec Ultima. A SPB-octyl (30m x 0.25mm id; 0.25 µm film) capillary column from Supelco was used for separation. Calibration standards (CS-0.2 through CS-5) were purchased from Cambridge Isotope Laboratories (encompassing a calibration range of 0.2 to 2000 ng/ml). Using PFK and molecular leak, the mass spectrometer was tuned to a resolving power ≥10,000 (10% valley). Calibration standards were injected (1µl) into an on-column injector by a CTC A200SE autosampler. GC conditions were as follows:

Interface temperature: 270ºC

Initial temperature: 70ºC

Initial time: 3 minutes

Temperature Program:

70-120 ºC@ 10 ºC/minute

20-280 ºC @ 3 ºC/minute

Final time: 22 minutes

Data were processed using OPUSquansoftware (Micromass) version 3.6.

Results and Discussion

Manufacturers advertise the Autospec Ultima as having a dynamic range of over five orders of magnitude[2]. Method 1668A calibration curve, when using CS-0.2 through CS-5, encompasses this entire range. The area counts typically range from $1x10^4$ in CS-0.2 to $1x10^9$ for most analytes in the CS-5. However, all the analytes in the method do not respond equally due to differences in chromatographic behavior, ionization, molecular ion abundance, and isotope patterns. Therefore, the method actually has an even greater range, making it difficult to make accurate assessments at the extreme ends of the instrument's dynamic range. When the instrument is cleaned and tuned for maximum sensitivity, some of the peaks often saturate in the CS-5 solution. This saturation distorts the CS-5 response factors (RFs). The distorted RFs skew the average RF which is applied in sample concentration calculations to all sample peaks, the majority of which are well within the dynamic range of the instrument.

Table 1 compares the RF values over the calibration range and compares the %RSD of the curve with the undiluted CS-5 to that of the curve with the CS-5 diluted by a factor of three. Many of the peaks in the undiluted CS-5 saturated and an estimated maximum possible concentration (EMPC) calculation (which uses the peak area of an ion with the least amount of saturation and the theoretical ratio) was used to give a better indication of peak area for calculating RFs. In doing this, the 20% RSD method criteria was exceeded by some of the analytes. Some of the secondary ions that were used to calculate the primary ion area and then the RF were close to saturation as well, skewing the RFs artificially low. The dilution clearly brings the peak area into the dynamic range of the instrument and therefore reduces the number of peaks that did not meet the ratio criteria, which also reduces the variability in the RFs across the calibration range. In addition, average RFs are more representative of analytes well within the instruments' dynamic range.

The principle of isotope dilution uses a ratio of the native analyte to its corresponding internal standard. If some of the native analyte is lost through the preparation process the internal standard experiences a proportional loss allowing quantification of the native analyte in the original sample at the point of internal standard introduction. The calibration is based on the range of the ratios of concentrations between the native analyte and internal standard, not the range of native analyte concentrations alone. Method 1668A calibration solutions cover the native to internal standard ratio range of 0.002:1 in the CS-0.2 to 20:1 in the CS-5. Dilution of the CS-5 solution will not change the ratio of the native to internal standard and is analogous to quantification at reduced sample preparation recovery. We have demonstrated this by analyzing a broad series of dilutions to the CS-5 standard. Table 2 shows that stable RFs are obtained with dilutions up to a factor of 300. However, diluting the CS-5 solution by a factor of three or four is sufficient to bring the peak areas within the dynamic range of the mass spectrometer, making the RF values more representative of peaks within the dynamic range of the instrument.

Table 1. Comparison of Response Factors using diluted vs. undiluted CS-5 solution.

	Standard Concentration (ng/ml)								
PCB	**0.2** CS-0.2 RF	**1** CS-1 RF	**5** CS-2 RF	**50** CS-3 RF	**400** CS-4 RF	**2000** CS-5 RF	**666.7** 1:3 CS-5 RF	**%RSD** w/ CS-5	**%RSD** w/ 1:3 CS-5
1	1.34	1.33	1.30	1.36	1.35	0.83 s	1.30	16	2
3	1.31	1.28	1.31	1.33	1.32	0.79 s	1.26	17	2
4	1.87	1.39	1.33	1.35	1.38	1.16	1.40	17	14
19	1.17	1.25	1.25	1.35	1.33	1.17	1.33	6	5
15	0.99	1.01	1.06	1.09	1.07	0.56 s	1.08	21E	4
54	1.47	1.28	1.28	1.38	1.38	1.30	1.37	6	5
104	1.35	1.30	1.24	1.27	1.26	1.17	1.27	5	3
37	0.96	0.96	0.97	1.00	1.01	0.52 s	1.02	21 E	3
155	1.08	1.13	1.15	1.17	1.20	1.11	1.19	4	4
81	1.05	1.05	1.05	1.07	1.10	0.69 s	1.08	15 E	2
77	1.02	0.98	1.00	1.03	1.06	0.66 s	1.05	15	3
123	1.01	0.92	0.94	1.00	1.00	0.79 s	0.99	9	4
118	1.07	0.91	0.96	1.01	1.00	0.77 s	1.02	10	5
188	1.02	1.07	1.11	1.17	1.16	1.00	1.14	7	5
114	1.03	0.95	0.94	0.98	0.99	0.88 s	0.98	5	3
105	1.04	0.93	0.94	1.00	0.99	0.77 s	0.98	10	4
126	1.03	0.88	0.91	0.95	0.96	0.75 s	0.95	10	5
202	0.90	0.95	0.94	0.96	0.98	0.97	1.05	3	5
167	0.92	0.86	0.89	0.93	0.93	0.70 s	0.93	10	3
156/157	0.91	0.90	0.93	0.97	0.97	0.60 s	0.96	16	3
169	1.09	0.86	0.85	0.89	0.90	0.76s	0.90	12	10
208	1.07	0.98	0.98	1.02	1.07	0.99	1.06	4	4
189	0.84	0.74	0.75	0.78	0.79	0.69 s	0.79	7	5
205	1.24	1.01	0.98	1.04	1.04	1.05 s	1.06	9	9
206	0.97	1.03	0.99	1.06	1.06	1.03	1.07	4	4
209	0.91	0.79	0.77	0.82	0.82	0.76	0.82	7	6

s=Saturated peaks, RFs attained by using theoretical ratios and EMPC calculations.

E= Exceeds 20% Method 1668A %RSD criteria

Table 2. Response Factors from CS-5 Dilutions

PCB	No Dilution	1:4	1:10	1:30	1:100	1:300	Avg RF	%RSD
1	0.91	1.20	1.23	1.29	1.27	1.20	1.18	12
3	0.90	1.13	1.20	1.19	1.22	1.30	1.16	12
4	1.17	1.17	1.34	1.29	1.29	1.40	1.28	7
19	1.18	1.23	1.15	1.24	1.40	1.09	1.22	9
15	0.94	1.00	0.99	1.04	1.03	0.99	1.00	4
54	1.16	1.30	1.34	1.38	1.40	1.63	1.37	11
104	1.18	1.17	1.19	1.21	1.16	1.13	1.17	2
37	1.05	1.01	1.05	1.06	1.04	1.11	1.05	3
155	1.25	1.06	1.07	1.06	1.09	1.07	1.10	7
81	0.97	1.00	1.02	1.01	1.02	1.00	1.00	2
77	0.93	0.94	0.95	0.96	0.98	0.96	0.95	2
123	0.98	0.98	1.00	1.01	1.01	1.00	1.00	1
118	0.99	1.01	1.00	1.05	0.99	0.99	1.01	2
188	1.04	1.24	1.25	1.33	1.34	1.33	1.25	9
114	0.98	0.98	0.99	0.99	1.00	1.00	0.99	1
105	0.97	0.98	1.00	1.01	1.01	1.01	1.00	2
126	0.95	0.95	0.95	0.96	0.94	0.96	0.95	1
167	1.07	1.07	1.09	1.10	1.12	1.14	1.10	2
156/157	1.02	1.11	1.10	1.12	1.12	1.12	1.10	4
169	1.04	1.05	1.06	1.07	1.08	1.04	1.05	1
208	0.97	1.00	1.01	1.00	1.00	0.98	0.99	2
189	0.96	0.99	0.99	1.01	0.99	0.98	0.99	2
205	1.02	1.03	1.06	1.07	1.03	1.02	1.04	2
206	0.99	1.00	1.02	1.03	1.02	1.03	1.01	2
209	0.98	0.99	1.01	1.02	1.02	1.03	1.01	2

Data is not available for PCB 202, as it went outside of the retention time window for some of the samples.

Samples containing PCB levels at or above the CS-4 and CS-5 levels need to be monitored carefully for saturating peaks and area counts outside of the dynamic range of the instrument. Generally this is not an issue because there is less than 100% recovery of the analytes during preparation. This loss is enough to keep the area counts within the dynamic range. However, if sample peak areas are either above that of the CS-5 or are saturated, the samples should be diluted as stated in M1668A Section 17.5.

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