Method Development and Validation for the Measurement of Hexachlorobutadiene and Chlorobenzenes in Trichloro- and Tetrachloro-ethylene

Chung Chiu[1], Anivis Sanchez[1], Mylaine Tardif[1], Gary Poole[1], Ying Jiang[1], Angela Marklew[1]

[1]ETC, Environment Canada, Ottawa

Introduction

Under the Canadian Environment Protection Act (CEPA, 1999), a substance is classified as CEPA-toxic if the substance is found to be toxic, persistent, bioaccumulative, and predominantly anthropogenic. CEPA-toxic substances are targeted for virtual elimination. Environment Canada and Health Canada have declared hexachlorobutadiene (HCBD), tetrachlorobenzenes (T_4CB), pentachlorobenzene (P_5CB) and hexachlorobenzene (HCB) as CEPA-toxic and have proposed their addition to the Virtual Elimination List.[1-5]

This paper summarizes the analytical processes for the development and validation of a reference method for measuring HCBD and CBs in chlorinated solvents, namely trichloroethylene (C_2HCl_3) and tetrachloroethylene (C_2Cl_4).

Method and Materials

Method Development

A performance-based reference method based on the use of isotopically-labelled surrogates and GC-MS with the selected ion monitoring technique was developed.

Sample Preparation: A 10mL sample size of the chlorinated solvent was spiked with an isotopically labelled surrogate solution containing 500ng of each $^{13}C_4$-HCBD, $^{13}C_6$-1,2,3,4-T_4CB, $^{13}C_6$-P_5CB and $^{13}C_6$-HCB. To achieve low, medium and high levels of target analytes, selected samples were fortified with known

amounts of HCBD, all three T_4CB isomers, P_5CB and HCB. The sample solvent volume was reduced to ~ 2mL by rotary evaporation before Florisil sample cleanup. While sample cleanup is recommended for various solvent grades, it may not be needed for high purity solvent grades. The samples were concentrated to approximately 0.9mL under a gentle stream of nitrogen and 0.1 mL of d_{10}-acenaphthene at 10ug/mL was added as the recovery standard. Samples were made up to a final volume of 1.0mL with isooctane and transferred to a 1.5mL amber vial with a Teflon lined cap and were stored in the refrigerator until HRGC/LRMS analysis.

HRGC/LRMS Analysis: A 1µL splitless (270^{o}C) injection was made to a HP6890 GC interfaced directly to HP5973 Mass Selective Detector using a HP7673A autosampler. The GC was equipped with a 10 m x 0.5 mm deactivated fused silica pre-column and a 30.0 m DB-XLB analytical column with 0.25 mm ID, a film thickness of 0.25 µm. The GC temperature program was 50^{o}C for 2 min, 12^{o}C/min to 160^{o}C for 1 min, 30^{o}C/min to 270^{o}C for 4 min, at a flow rate of 2.0 mL/min.

Quantification: HRGC-LRMS analysis was conducted using electron impact ionization and selected ion monitoring (SIM) techniques in which a minimum of three ions were monitored for all native analytes, two characteristic ions for each of the surrogate analytes and one ion for the recovery standard, as displayed in **Table 1**. A typical total ion chromatogram is shown in **Figure 1**. The target analytes were quantified using the Internal Standard (Isotope Dilution) Quantification procedures. As a result, analyte concentrations were corrected for the surrogate recovery.

Method Validation

Various grade of C_2HCl_3 and C_2Cl_4 were purchased from different suppliers to determine the background levels of target analytes in these solvents. Samples were fortified with native analytes to achieve three concentration levels, low, medium and high (approximately 0.5, 50 and 300 ng/mL or higher respectively). The highest grade solvent was used to test the low (0.5ng/mL) level, whereas the lower grade solvent was used to test the other two levels.

A total of 12 batches of 10mL samples at each concentration level were processed and analyzed in duplicate during a 10-week period. A solvent blank and a cleanup blank were also analyzed with each batch of samples. Calibration checks were

performed with each batch of samples. The background level of target analytes attributed from reagents, glassware, and surrogate solution were assessed prior to the replicate analyses.

Table 1 Quantification Standard and Monitored Ions for Hexachlorobutadiene, Tetrachlorobenzene, Pentachlorobenzene and Hexachlorobenzene Analysis

Analyte	Quantification Ion (m/z)	Confirmation Ion (m/z)	Relative Intensities (%)
HCBD	224.8	189.8, 259.7	100, 38, 38
1,2,3,4-T_4CB	215.8	213.8, 178.9	100, 77, 17
1,2,3,5-T_4CB	215.8	213.8,178.9	100, 77, 17
1,2,4,5-T_4CB	215.8	213.8,178.9	100, 77, 17
P_5CB	249.8	247.8, 214.8	100, 62, 19
HCB	283.8	281.8, 248.8	100, 53, 25
$^{13}C_4$-HCBD	232.8	267.8	100, 67
$^{13}C_6$-T_4CB	221.9	223.9	100, 46
$^{13}C_6$-P_5CB	257.8	259.8	100, 32
$^{13}C_6$-HCB	291.9	293.8	100, 44
d_{10}-AE	164.1		100

Results and Discussion

Sensitivity: To determine the instrumental detection limit (IDL), HCBD and CBs native standards were diluted to various concentrations and analyzed. The IDL was the lowest concentration in which the three selected ions had a signal to noise ration of 3:1. The IDL was determined to be 0.5 pg per µL injection. Sample Detection Limit (DL) can then be estimated as 0.5 ng/sample on the basis of 1000µL final volume with 1 µL injection. This represents 0.05 ng/mL for a 10mL sample size. **Figure 2a-b** displays the selected ion chromatographs of the target analytes at 0.5pg.

Linearity: The linear dynamic range was established by using six levels of calibration standards with concentrations of HCBD and CBs ranging from 5ng/mL to 5000ng/mL (0.005-5.00µg/mL). Linearity over the working range was established when the coefficient of determination (R^2) for each analyte was ≥ 0.995. For a linear curve, the response factors (RFs) and relative response factors (RRFs) at different concentrations should remain constant. Curve linearity tended to decrease at concentrations above 5000ng/mL. The comparison of average RRFs from a 6-point calibration shown that the RSDs of the average RRFs for all analytes were well below 10%.

Repeatability and Recoveries: Over a 10-week period, 12 batches of repeat analysis of 10mL samples were analyzed in duplicates and statistical assessments were performed on the final results. **Table 2** displays the concentration mean, standard deviation (SD) and relative standard deviation (RSD) of each target analyte at each concentration level along with surrogate recoveries. Results indicate that as the concentration of the analytes increases, the SD increases. RSD measures the extent to which the results are spread around their average. The calculated RSD at each concentration level ranges from 0.98% to 23.24%. The general trend indicates that as the concentration level decreases, the RSD values increases. HCBD had the highest RSD (23.24%) value compared to the lowest value of 4.66% for HCB at the lowest concentration level. This may be due to the relatively high volatility of HCBD.

Mean surrogate recoveries for all analytes at three levels were very consistent between 71 and 88%. The RSD of the recoveries were in the range of 6 – 16%. Results suggested that the RSD for tetrachloroethylene (8.3-16.2%) is greater than trichloroethylene (6.3-8.8%) for all target analytes. This may be due to its higher boiling point (121^{o}C vs. 87^{o}C) which required longer period of time to reduce the final volume to less than 1 mL.

Accuracy: There is no standard reference material currently available for the testing of accuracy for this method. Two standard mixtures were purchased from suppliers and analyzed using the daily calibration standard. The discrepancy in concentrations for target analytes were under 10% except for 1,2,4,5- T_4CB with a deviation of 14%.

Interferences: Hexachlorobutene was found as an interference in lower grade trichloroethylene samples. Hexachlorobutene, having ion 179 in common with the

3rd qualifier ion of 1,2,3,5-T_4CB, resulted in the augmentation of the ion ratio. This interference, however, does not have a significant impact on quantification of target analyte.

Robustness: The robustness of the method was examined by deliberately changing some key operational conditions that could have an impact to the accuracy of results. Experimental data show that the concentration of the target analytes remains constant and accurate when the final volume is changed from 0.5 mL to 2 mL or the injection is varied from 0.5 µl to 1.5 µl.

Conclusion

A sensitive and reliable analytical method suitable for the quantitative determination of Hexachlorobutadiene and Chlorobenzenes in chlorinated solvents was established. 12 batches of fortified HCBD and CBs samples at various levels yielded a RSD range of 0.98% - 23.24%, with surrogate recoveries ranging from 70.92 – 87.71%. The 23.24% RSD for HCBD was considered to be due to its high volatility. The method was written in a performance-based format. There is a prerequisite to validate analytical performance of the method before the samples are processed. Reliable results are expected when the reference method is followed and all the quality assurance criteria are met.

References

1. Environment Canada/Health Canada. 2001. *Canadian Environmental Protection Act, 1999 - Priority Substance List Assessment Report - Hexachlorobutadiene*.
2. Environment Canada/Health and Welfare Canada. 1993. *Canadian Environmental Protection Act - Priority Substance List Assessment Report - Tetrachlorobenzene*.
3. Environment Canada/Health and Welfare Canada. 1993. *Canadian Environmental Protection Act - Priority Substance List Assessment Report - Pentachlorobenzene*.
4. Environment Canada/Health and Welfare Canada. 1993. *Canadian Environmental Protection Act - Priority Substance List Assessment Report - Hexachlorobenzene*.
5. C.H. Chiu, A. Sanchez and M. Tardif, *Level of Quantification for Measuring Chlorobenzenes in Chlorinated Solvents*, 2002, Organohalogen Compounds, 55, 135-138.

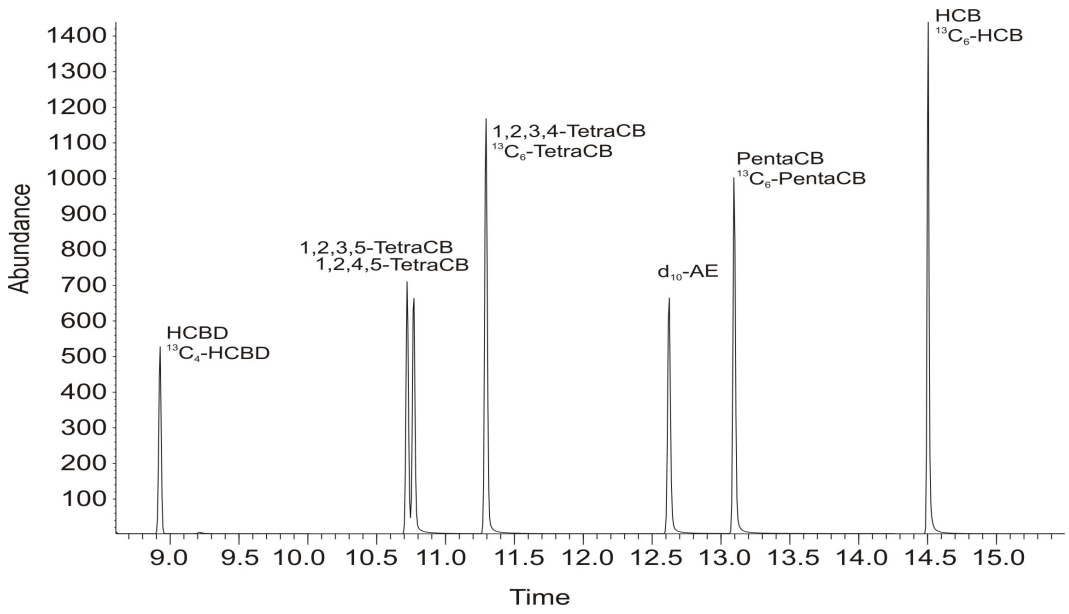

Figure 1 **Total Ion Chromatograph of Hexachlorobutadiene, Tetrachlorobenzenes, Pentachlorobenzene, Hexachlorobenzene and Surrogates at 0.5µg/mL**

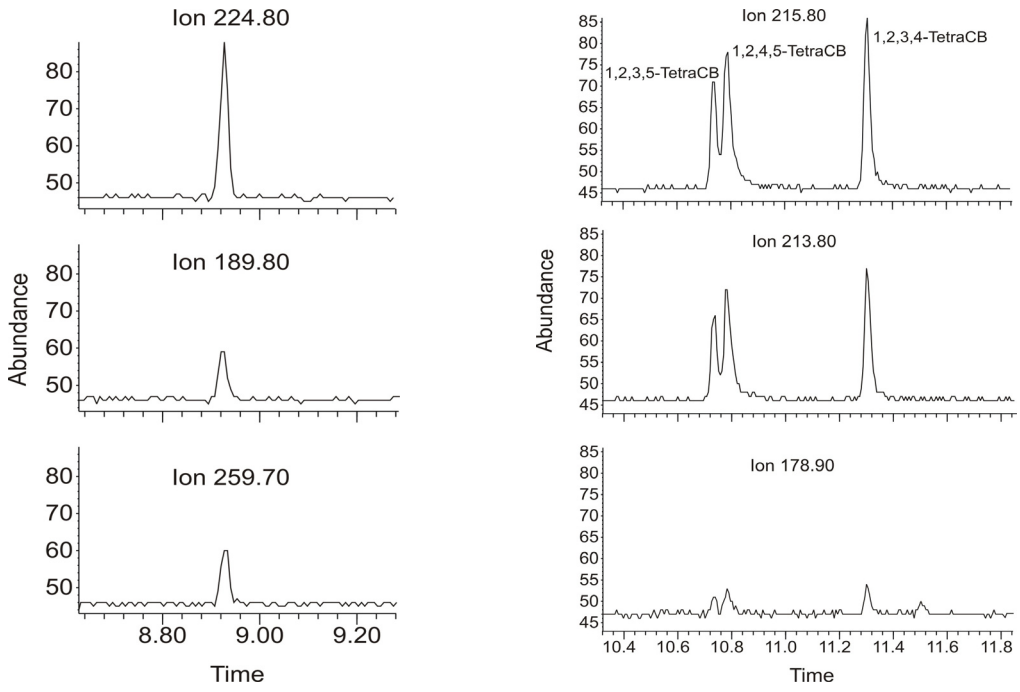

Figure 2 **Selected Ion Chromatograph of (a) Hexachlorobutadiene and (b) Tetrachlorobenzenes at 0.5pg**

Table 2 Mean Concentrations, SD and RSD for Repeat Analysis of C_2HCl_3 and C_2Cl_4 Samples

Solvent	Level	Replicate	HCBD			P_5CB			HCB		
			Mean	SD	RSD	Mean	SD	RSD	Mean	SD	RSD
		n	µg/sample	µg/sample	%	µg/sample	µg/sample	%	µg/sample	µg/sample	%
C_2HCl_3	Low	24	0.00583	0.00072	12.3	0.00570	0.00034	5.9	0.00536	0.00025	4.66
	Med.	24	0.52764	0.01686	3.20	0.49888	0.01479	3.0	0.49383	0.00886	1.79
	High	24	3.00188	0.06734	2.24	2.99740	0.07615	2.5	2.88157	0.06002	2.08
C_2Cl_4	Low	24	0.01383	0.00321	23.24	0.00690	0.00068	9.8	0.00616	0.00055	8.95
	Med.	23	0.53780	0.00645	1.20	0.49303	0.00711	1.4	0.49686	0.00608	1.22
	High	24	9.12357	0.15524	1.70	2.82040	0.04503	1.6	2.82353	0.03500	1.24
^{13}C-Recovery:			**%**	**^{13}C-HCBD**		**%**	**^{13}C-P_5CB**		**%**	**^{13}C-HCB**	
C_2HCl_3	Low	24	82.52	6.29	7.63	86.98	5.95	6.84	87.71	5.65	6.45
	Med.	24	79.88	5.99	7.50	82.47	5.92	7.17	82.60	5.18	6.27
	High	24	81.06	7.43	9.17	79.55	7.00	8.79	83.03	6.91	8.32
C_2Cl_4	Low	24	74.25	8.62	11.61	83.08	9.02	10.86	82.58	8.45	10.23
	Med.	23	70.92	11.51	16.23	81.53	6.99	8.57	82.06	6.78	8.27
	High	24	87.18	11.12	12.76	77.65	9.05	11.65	81.86	9.09	11.10

Solvent	Level	Replicate	1,2,3,5-T_4CB			1,2,4,5-T_4CB			1,2,3,4-T_4CB		
			Mean	SD	RSD	Mean	SD	RSD	Mean	SD	RSD
		n	µg/sample	µg/sample	%	µg/sample	µg/sample	%	µg/sample	µg/sample	%
C_2HCl_3	Low	24	0.00550	0.00047	8.59	0.00505	0.00051	10.08	0.00528	0.00050	9.40
	Med.	24	0.49176	0.01549	3.15	0.46367	0.01478	3.19	0.54758	0.02183	3.99
	High	24	2.81263	0.05770	2.05	2.67368	0.06206	2.32	3.06470	0.07465	2.44