Organohalogen Compounds, Volume 60, Pages 138-141 (2003)

ANALYSIS OF STOCKHOLM CONVENTION PRIORITY POPS WITH HIGH SENSITIVITY AND HIGH QUALITY USING ^{13}C-ISOTOPE STANDARDS AND HRGC-HRMS: CLEANUP AND ANALYSIS

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Introduction

The Stockholm Convention (SC) is a global treaty to protect human health and the environment from persistent organic pollutants (POPs). POPs are organic chemicals that remain intact in the soil, sediment as well as several segments of environment for prolonged periods, become ubiquitously distributed over global scale, they accumulate in the lipids of living things and are highly toxic to humans and wildlife. POPs circulate globally and can cause damage wherever they travel. The significant awareness and requirement of monitoring of 12 priority POPs has been increased after SC meeting conducted in May 2001. In this meeting, SC proposed aldrin, dieldrin, endrin, toxaphene, mirex, haptachlor, chlordane compounds, hexachlorobenzene, DDT compounds, PCBs, PCDDs and PCDFs as priority POPs that should monitored over global terms. Establishment of high-sensitivity and high quality analysis of POPs using high-resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) seems to essential in future analytical chemistry. The HRGC-HRMS has several advantage of quantifying trace level of toxic contaminants in various sample matrixes and also give precise analytical data up to femtogram levels (fg/g). The ^{13}C-Isotope standards as internal standard for the POPs analysis have further advantage in HRGC-HRMS.

Among 12 POPs proposed by "SC", except 2 compounds such as toxaphene and mirex has been omitted in this study due to its no usage and lesser environmental occurrence in Japan. Consequently, in addition to the SC-priority list with inclusion of HCH-isomers, all SC-POPs were established in this study. PCDDs, PCDFs and dioxin-like PCBs analysis, the traditional method has been implied although the extraction procedure was same for all POPs. It should be worth stating that some compounds such as aldrin, dieldrin, endrin and heptachlor get easily decomposed when purifying PCDD/DFs and PCBs. Therefore, special and separate clean-up method has been proposed for organic pesticides and successfully carried out in this study.

Materials and Methods

Standard solutions: For the precise and purity work with improved detection limits, we proposed the extraction standards, internal standards and syringe spike standards with carbon-labeled isotope for the analysis of variety of the environmental as well as biological samples. In this regard, for pesticides, $^{13}C_6$-α-HCH, $^{13}C_6$-β-HCH, $^{13}C_6$-γ-HCH, $^{13}C_6$-δ-HCH, $^{13}C_6$-hexachlorobenzene, $^{13}C_{10}$-heptachlor, $^{13}C_{12}$-aldrin, $^{13}C_{12}$-dieldrin, $^{13}C_{12}$-endrin, $^{13}C_{10}$-heptachlor epoxide, $^{13}C_{10}$-oxychlordane, $^{13}C_{10}$-trans-chlordane, $^{13}C_{10}$-trans- nonachlor, $^{13}C_{12}$- p,p'-DDE, $^{13}C_{12}$-

o,p'-DDT and $^{13}C_{12}$- p,p'-DDT internal standards and 4-HCH isomer, hexachlorobenzene, 6-DDT compounds, 7-chlordane compounds, 3-drin compounds native standard solutions were purchased from Cambridge Isotope Laboratories (CIL), Accustandard, Supelco, and Wako pure chemical as shown in Table 1. The sampling or extraction isotope standards were cis-nonachlor, o,p-DDE and δ-HCH while, syringe spike was ^{13}C-2,3',4',5-TeCB(IUPAC#70). For the calculations, the carbon-isotope solutions used for the corresponding native peaks (shown in Table 1) have been established and discussed. With the new native and internal standard materials, the calibration curve was performed with double monitor ions and the mass ranges were established (Table 2).

Table 1. Native and internal standards of SC-priority POPs and calibration curve concentrations.

Native Standard	CS1 (pg/μL)	CS2	CS3	CS4	CS5	CS6	Internal Standard	CS1- to CS6 (pg/μL)
α-HCH	1000	200	40	10	2	0.4	$^{13}C_6$-α-HCH	40
β-HCH	1000	200	40	10	2	0.4	$^{13}C_6$-β-HCH	40
γ-HCH	1000	200	40	10	2	0.4	$^{13}C_6$-γ-HCH	40
δ-HCH	1000	200	40	10	2	0.4	$^{13}C_6$-δ-HCH *	40
HCB	1000	200	40	10	2	0.4	$^{13}C_6$-HCB	40
Aldrin	1000	200	40	10	2	0.4	$^{13}C_{12}$-Aldrin	40
Dieldrin	1000	200	40	10	2	0.4	$^{13}C_{12}$-Dieldrin	40
Endrin	1000	200	40	10	2	0.4	$^{13}C_{12}$-Endrin	40
Heptachlor	1000	200	40	10	2	0.4	$^{13}C_{10}$-Heptachlor	40
Heptachlor epoxide	1000	200	40	10	2	0.4	$^{13}C_{10}$-Heptachlor epoxide	40
Oxychlordane	1000	200	40	10	2	0.4	$^{13}C_{10}$-Oxychlordane	40
trans-Chlordane	1000	200	40	10	2	0.4	$^{13}C_{10}$-trans-Chlordane	40
cis-Chlordane	1000	200	40	10	2	0.4		
trans-Nonachlor	1000	200	40	10	2	0.4	$^{13}C_{10}$-trans-Nonachlor	40
cis-Nonachlor	1000	200	40	10	2	0.4	$^{13}C_{10}$-cis-Nonachlor *	40
o,p'-DDE	1000	200	40	10	2	0.4	$^{13}C_{12}$-o,p'-DDE *	40
p,p'-DDE	1000	200	40	10	2	0.4	$^{13}C_{12}$-p,p'-DDE	40
o,p'-DDD	1000	200	40	10	2	0.4		
p,p'-DDD	1000	200	40	10	2	0.4		
o,p'-DDT	1000	200	40	10	2	0.4	$^{13}C_{12}$-o,p'-DDT	40
p,p'-DDT	1000	200	40	10	2	0.4	$^{13}C_{12}$-p,p'-DDT	40
							$^{13}C_{12}$-2,3',4',5-TeCB **	40

* sampling or Extraction spike
** syringe spike

Clean-up: The clean-up was performed with silicagel, florisil, H_2SO_4-treatment, 22%-H_2SO_4-silicagel, 44%-H_2SO_4-silicagel and $AgNO_3$-silicagel with hexane (HEX), dichloromethane (DCM) and diethylether (DEE) as elution solvents. Since the acid (H_2SO_4 and $AgNO_3$)-impersed silicagel degraded some POPs like endrin, heptachlor and aldrin, only activated (5 h with 10 g florisil for each column) florisil was used with HEX and HEX:DCM as elution solvents. The HEX alone seems to good separator solvent but for complete separation some % of DCM should be used. For more dirty samples dimethyl sulfoxide (DMSO) treatment was performed in order to remove fat

and non-target chemicals. The overall results obtained with these cleanup conditions have been discussed.

Table 2. The monitor ions of POPs analyzed in this study using HRGC-HRMS.

POPs	M	M+2	M+4
HCH (M-HCl_2)	180.9379	182.9349	
HCB		283.8102	285.8073
Heptachlor (M-C_5H_5Cl)	271.8102	273.8072	
Aldrin (M-C_5H_6Cl), Dieldrin,Endrin (M-C5H6ClO)		262.8570	264.8541
Heptachlor epoxide (M-Cl)		352.8442	354.8413
Oxychlordane (M-Cl)		386.8053	388.8024
Chlordane (M-Cl)		372.8260	374.8231
Nonachlor (M-Cl)		406.7870	408.7841
DDE (M-Cl_2)	246.0003	247.9975	
DDD (M-$CHCl_2$), DDT (M-CCl_3)	235.0081	237.0053	
$^{13}C_6$-HCH (M-HCl_2)	186.9580	188.9550	
$^{13}C_6$-HCB		289.8303	291.8273
$^{13}C_{10}$-Heptachlor (M-C_5H_5Cl)		276.8269	278.8240
$^{13}C_{12}$-Aldrin (M-C_5H_6Cl), $^{13}C_{12}$-Dieldrin,Endrin (M-C_5H_6ClO)		269.8804	271.8775
$^{13}C_{10}$-Heptachlor epoxide (M-Cl)		362.8777	364.8748
$^{13}C_{10}$-Oxychlordane (M-Cl)		396.8387	398.8358
$^{13}C_{10}$-Chlordane (M-Cl)		382.8595	384.8565
$^{13}C_{10}$-Nonachlor (M-Cl)		416.8205	418.8175
$^{13}C_{12}$-DDE (M-Cl_2)	258.0405	260.0376	
$^{13}C_{12}$-DDT (M-Cl_3)	247.0483	249.0454	
$^{13}C_{12}$-2,3',4',5-TeCB(#70)	301.9626	303.9597	

Quantification and Identification: The Micromass Autospec Ultima high-resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) HP-6890 Series GC system was used and their conditions for PCDD/DFs, PCBs and organic pesticides were conditioned separately. Both DB-5MS and DB-17HT was used for analysis due to different polarity of phenyl methyl silicone in according columns. The analysis was also extended with performing analysis of 100 fg/ul standard solutions and their corresponding concentrations were shown in Table 3. The instrument detection limit (IDL) and instrument quantification limit (IQL) was set with 3-times and 10-times of standard deviation(STDEV) of this solutions that set to analyze in HRGC-HRMS. The double ion detected the concentrations up to 7.8 to 54 (IDL) and 26-180 (IQL) fg/injection. The p,p'-DDT was detection at most while endrin shows least.

Results and Discussion

General: Based on the new standard solutions, the calibration curve was well fit with the corresponding concentrations. The relative response factor (RRF) and relative standard deviation (RSD) in double ion monitoring window for most of the POPs were well correspond to the theoretical values (RRF; 0.915-1.487 and RSD;0.9-7.1%). The results were obtained in DB-5 MS and DB-17HT columns in case compounds interfered in DB-5 column, the DB-17 HT results were

used. In DB-5 MS column heptachlor epoxide/oxychlordane and cis-nonachlor/p,p'-DDD were found to have broad mass range and thus response gets poor for separate peaks. While DB-17 HT column grouping showed clear and separate peaks for all POPs and thus we fixed DB-17HT for further analysis.

Cleanup and Separation: The cleanup and separation conditions were established as that extracted environmental samples can be fractionated directly with florisil with 20% DCM;HEX as pre-eluant and DCM as post eluant. Nevertheless, wildlife samples needed to be cleaned with DMSO further. DMSO cleaned sample that fractionated with silicagel and florisil seems to most appropriate method, whereas, sulfuric acid cleaned samples degrades dieldrin, endrin, heptachlor epoxide and aldrin while silver nitrate degrades heptachlor. Therefore DMSO and florisil method were selected for further analysis purpose and we conducted the analysis of environmental samples and variety of biological samples from Japan. The analytical results of 26 biological samples suggested that 0.05 ng/g detection limits for HCHs, chlordanes, HCB, aldrin, DDTs, DDDs, 0.2 ng/g for dieldrin, endrin, oxychlordane, 0.1 ng/g for o,p-DDE and p,p'-DDE on wet biological samples.

Table 3. Quantification limits of HRGC-HRMS with standard solutions (fg).

	Average Conc. (fg) n=6		STDEV		IDL* (fg)		IQL** (fg)	
Chemicals	Ion1	Ion2	Ion1	Ion2	Ion1	Ion2	Ion1	Ion2
a-HCH	199	199	4.4	4.9	13	15	44	49
b-HCH	208	212	9.4	7.1	28	21	94	71
g-HCH	200	203	6.9	9.3	21	28	69	93
d-HCH	200	201	8.8	7.0	26	21	88	70
HCB	203	202	3.1	6.2	9.3	19	31	62
Aldrin	213	217	8.9	7.3	27	22	89	73
Dieldrin	208	199	12	8.7	36	26	120	87
Endrin	210	210	18	18	54	54	180	180
Heptachlor	211	213	8.6	9.3	26	28	86	93
Heptachlorepoxide	200	203	9.6	9.0	29	27	96	90
Oxychlordane	214	212	16	7.8	48	23	160	78
trans-Chlordane	203	201	6.7	9.3	20	28	67	93
cis-Chlordane	192	191	8.9	12	27	36	89	120
trans-Nonachlor	205	202	12	12	36	36	120	120
cis-Nonachlor	201	201	5.2	4.2	16	13	52	42
o,p'-DDE	210	211	5.3	6.0	16	18	53	60
p,p'-DDE	204	208	8.9	9.1	27	27	89	91
o,p'-DDD	224	225	7.8	13	23	39	78	130
p,p'-DDD	192	198	4.7	9.8	14	29	47	98
o,p'-DDT	198	209	3.7	7.8	11	23	37	78
p,p'-DDT	199	204	5.2	2.6	16	7.8	52	26

*Instrument detection limit, **Instrument quantification limit

It is worth indicating that HEX seems to retain HCB and aldrin with less than 10% during hexane and DMSO partitioning and thus DMSO analytical results used with caution. Other results showed that only sulfuric acid treatment influenced dieldrin and endrin recovery, while, sulfuric acid with silicagel influenced aldrin and heptachlor epoxide. Eventually, for the dioxins, the traditional analytical method was adopted and the analytical conditions were established. Further, several aspects were discussed with regard to suitability of high quality analysis of SC priority POPs.