Organohalogen Compounds, Volume 60, Pages 29-32 (2003)

Dioxin analysis in human serum of small sized sample by using concentration at the inlet of the GC capillary column for 100 µL large volume injection method

Kimiyoshi Kitamura [1], Yoshiyuki Takei [2], Jae-Won Choi[1], Shunji Hashimoto[1], Hiroyasu Ito[1] and Masatoshi Morita [1]

[1]National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-8506, Japan
[2] GL Sciences, 237-2 Sayamagahara, Iruma, Saitama, 358-0032, Japan

Introduction

In recent years the concern for the effects of dioxins (PCDDs/Fs and Co-PCBs) on humans has grown, blood samples are frequently used to investigate human exposure to them. The determination of dioxins in human blood or serum with high resolution gas chromatograph - high resolution mass spectrometry (HRGC-HRMS) for ultra trace analysis is performed with the mass spectral resolution maintained at 10 000 and using a capillary column with a low polarity stationary phase. The sensitivity which can be obtained with this method is generally S/N=5-10 with injection amounts of 10 fg. One method to improve performance and increase sensitivity is to increase the injection volume into the HRGC-HRMS. A blood sample of 50-100 g (serum; 25-50 g) is needed to achieve a TCDD determination limit of about 1 pg/g lipid. This produces a final sample solution of 20 µL and a volume of 2 µL is injected into the HRGC-HRMS. Sampling of 100 g of blood including dual measurement has generally been adopted. Concerning the investigation of the blood (serum) sample, the reduction of the sample weight is beneficial for the subjects. We examined the potential of the accurate determination of dioxins by using 5 g serum. There have been some publications on about 10 µL large volume injection methods (LVI) for dioxin analysis[1-3]. We aimed to apply concentration at the inlet of the GC capillary column for LVI (At-column) to inject 100 µL of the 110 µL final sample solution into the HRGC-HRMS for dioxins analysis. A serum sample of 25 g was analyzed using conventional methods as the control. Our aim was to increase the sensitivity of the HRGC-HRMS, without increasing noise, by injecting the majority of the extract from a small sized blood sample.

Material and methods

Extraction

The Serum used was a reference material for interlaboratory calibration studies made by the National Institute for Environmental Studies (NIES, Japan). A Serum sample of 5 g was transferred to a 50 ml centrifuge-tube, and 2.2 pg of $^{13}C_{12}$-PCDDs/Fs (4.4 pg of $^{13}C_{12}$-OCDD/F) and 22 pg of $^{13}C_{12}$-Co-PCBs were added. The serum was shaken for 30 min. after the addition of 3 ml of saturated ammonium sulfate solution and 12 ml of 25%(v/v)-ethanol-hexane. The upper hexane layer was passed through a column containing 10 g anhydrous Na_2SO_4. The lower layer was extracted with 10 ml of hexane and then passed through the same anhydrous Na_2SO_4 column twice. The combined hexane fraction obtained was concentrated to about 2 ml by a rotary evaporator and then dried by standing at room temperature in a desiccator. The extracted lipid was then weighed.

A Serum sample of 25 g was transferred to a 250 ml centrifuge-tube, and 20 pg of $^{13}C_{12}$-PCDDs/Fs (40 pg of $^{13}C_{12}$-OCDD/F) and 220 pg of $^{13}C_{12}$-Co-PCBs were added. The same procedure as described above was carried out except five times the volume of solvent was used.

Cleanup and analysis

The 5 g serum samples were cleaned up by passing through a multi-layer column composed of 0.5 g of silica gel, 1 g of 2%(w/w) KOH-silica gel, 0.5 g of silica gel, 2 g of 44%(w/w) H_2SO_4-silica gel, 2 g of 22%(w/w) H_2SO_4-silica gel, 0.5 g of silica gel, 1 g of 10%(w/w) $AgNO_3$-silica gel, 0.5 g silica gel and 6 g anhydrous Na_2SO_4 in order from the top to the bottom layer. After elution with 60 ml of n-hexane the specimen was evaporated to an approximate volume of 2 ml. The concentrate was applied to an active carbon-dispersed silica gel column composed of 0.6 g of anhydrous Na_2SO_4, 0.2 g of active carbon-dispersed silica gel and 0.6 g of anhydrous Na_2SO_4, eluted with 10 ml of 25% (v/v) dichloromethane/n-hexane, and then eluted with 50 ml of toluene into a pear shaped pointed bottom flask. The toluene fraction was evaporated to approximately 1 ml and the internal standard, spiked in toluene, was added. The sample was evaporated to dryness under a nitrogen gas flow and then made up to approximately 110 µL with toluene. The sample of 100 µL in a final sample solution of 110 µL was injected into the Optic2 injector equipped with an At-column kit to analyze by HRGC-HRMS.

The 25 g serum samples were cleaned up by passing through a multi-layer column composed of 0.9 g of silica gel, 3 g of 2%(w/w) KOH-silica gel, 0.9 g of silica gel, 4.5 g of 44%(w/w) H_2SO_4-silica gel, 6 g of 22%(w/w) H_2SO_4-silica gel, 0.9 g of silica gel, 3 g of 10%(w/w) $AgNO_3$-silica gel and 6 g anhydrous Na_2SO_4 in order from the top to the bottom layer. After elution with 200 ml of n-hexane the specimen was evaporated to a volume of approximately 2 ml. The concentrate was applied to an active carbon-dispersed silica gel column composed of 3 g of anhydrous Na_2SO_4, 0.5 g of active carbon-dispersed silica gel and 3 g of anhydrous Na_2SO_4, eluted with 100 ml of 25% (v/v) dichloromethane/n-hexane, and then eluted with 100 ml of toluene into a pear shaped pointed bottom flask. The toluene fraction was evaporated to about 1 ml and then transferred to a pointed bottom test tube. The internal standard, spiked in n-nonane, was added. The solvent was removed with a nitrogen gas flow and then made up to a volume of approximately 20 µL in n-nonane. The sample of 2 µL in a final sample solution of 20 µL was injected into the injector to analyze by HRGC-HRMS.

The HRGC/HRMS analysis was performed on a JMS-700 high resolution mass spectrometer (JEOL, Japan) coupled to a 6890 series gas chromatograph (Agilent, USA). The sample solution was injected into the HP6890/JMS-700 equipped with an Optic 2 large volume sample injection system (Atas GL, Japan). The conditions used by the Optic 2 large volume injection system equipped with At-column concentration and HRGC/HRMS are shown in Tables 1and 2 respectively.

Results and discussion

No significant difference was found in all congener levels measured in both the 5 g and 25 g samples except for 2,3,7,8-TCDF and any congeners of ND in 25 g, and these RSDs were under 10% (Table 3). The recovery of 2,3,7,8-TCDD and 2,3,7,8-TCDF in serum sample of 5 g was 61 and 63%, respectively. It was lower than that of the 25 g sample. TCDD/F congeners are easily volatilized in comparison with Pe-OCDD/F. Moreover, there is less matrix in a serum sample of 5 g than that of 25 g. Therefore, TCDD/F in serum samples of 5 g had conditions where it was more easily volatilized during the pre-treatment. On the other hand, recoveries of other congeners in the serum sample of 5 g were 67-107%, which was almost the same as that of 25 g. 2,3,7,8-TCDF could be quantitated only with At-column concentration, but this congener had the highest RSD of 15%. The reason is that the absolute volume introduced onto the capillary column was more from the 5 g than the 25 g sample.

It is actually very difficult for an analyst to make a final sample solution of 20 µL compared with 100 µL. In the case of 20 µL final sample solution, it is sometimes volatilized even when it is kept stored in toluene for a while; therefore we need to change the solvent to n-nonane. This work is difficult for the analyst, if toluene completely volatilizes, recoveries of the semi-volatile lowly chlorinated congeners will decrease. On the other hand, it is relatively easy to make 100 µL of the final sample solution; the sample solution was evaporated in pear shape flask, and then concentrated to more than 100 µL with nitrogen, without transferring to a test tube. A final sample solution of greater than 100 µL has a lower risk of volatilization and increased peak abundance. The serum sample of 5 g using At-column concentration could be measured without a problem as the analytical At-column concentration value, compared with that of 25 g using splitless, and the procedure to make a final sample solution beyond 100 µL was easy for the analyst, so the problem of the sample being easily volatilized with the conventional amount of final sample solution, was reduced. Therefore, dioxin analysis using At-column concentration is the most useful method for serum analysis; it not only increases sensitivity but also decreases time taken by the analyst.

Table 1 The condition of injection of 100uL by using OPTIC 2 large volume injection system equipped with At-column concentration

Large volume injection system (OPTIC2, ATAS GL, Japan)					
Equilibration time	: 30sec	Purge Pressure	: 25kPa	Final Pressure	: 310kPa
Initial Temperature	: 105℃	Transfer Pressure	: 100kPa	End Time	: 49min
Ramp Rate	: 16℃/sec	Split Open Time	: 2.5min	Split Flow	: 20ml/min
Final Temperature	: 280℃	Transfer Time	: 3min	Vent Flow	: 100ml/min
Vent time	: Auto (level 10)	Initial Pressure	: 100kPa	Purge Flow	: 3ml/min

Table 2-1 The condition of HRGC

HRGC (6890 series GC system, Agilent, USA)
GC Pre-column * Deactivated Capillary Tubing (GL Sciences, Japan), 30cm×0.53mm ID
GC Capillary column
CP-Sil 8 CB low bleed/MS (Varian, USA), 60m×0.25mm ID, 0.25µm film thickness
Ramp of Oven Temp.
Large Volume Inj.
140℃(5℃min)-(15℃/min)-200℃(0min)-(3℃/min)-300℃(0min)-(5℃/min)-310℃(5min)
Splitless Inj.
Injection Port Temp. (260℃)
120℃(1.5℃min)-(15℃/min)-200℃(0min)-(3℃/min)-300℃(0min)-(5℃/min)-310℃(5min)

*For At-column concentration

References

1. Tanaka, K., Nojima, K., Makino, S. (1993) *J. Environ. Chem.*, 3, 514-515
2. Tajima, H., Shimamura, N., Takaoka, M., Nakazono, K. (1993) *J. Environ. Chem.*, 3, 446-447
3. Tajima, H., Shimamura, N., Aoki, M. (1993) Bunseki Kagaku, 42, 573-579

Table 2-2 The condition of HRMS

HRMS (JMS 700, JEOL, Japan)	
Ionizing current : 700 μA	Accelerating voltage : 10kV
Ionizing energy : 42 eV	Resolution : R>10,000 (10% valley)
Measurement Mass : Selected Ion Monitor (SIM) *using PFK	
$^{12}C_{12}$- and $^{13}C_{12}$- TCB, TCDD/F, PeCDD	M+, (M+2)+
$^{12}C_{12}$- and $^{13}C_{12}$- Pe-HxCB, PeCDF, Hx-OCDDF	(M+2)+, (M+4)+

* When the peak area ratio on the chromatogram for two monitored ions is almost equal to that of the standard substance and ion strength ratio is within ±15%, against those of values estimated with the isotopic abundance of chlorine atoms.

Table 3 Comparison between dioxin levels in 5 g serum using At-column concentration and 25 g serum using splitless

	pg/g lipid				Recovery (%)*	
	5g (At-column)		25g (Splitless)		5g	25g
Compounds	Mean	RSD(%)	Mean	RSD(%)	At-column	Splitless
PCDDs						
2,3,7,8-TCDD	2.2	4.7	2.3	2.3	61	88
1,2,3,7,8-PeCDD	7.6	4.6	7.9	1.3	84	95
1,2,3,4,7,8-HxCDD	6.3	0.94	6.3	1.0	100	85
1,2,3,6,7,8-HxCDD	61	0.59	62	2.6	95	75
1,2,3,7,8,9-HxCDD	7.5	2.6	7.8	0.74	103	90
1,2,3,4,6,7,8-HpCDD	56	1.3	56	1.1	89	70
OCDD	470	4.0	470	3.9	73	75
PCDFs						
2,3,7,8-TCDF	0.75	15	<0.25		63	76
1,2,3,7,8-PeCDF	(0.23)	21	<0.29		77	99
2,3,4,7,8-PeCDF	7.4	3.2	7.4	2.8	85	77
1,2,3,4,7,8-HxCDF	8.2	1.5	8.4	1.7	94	79
1,2,3,6,7,8-HxCDF	6.2	4.7	6.3	3.0	80	70
1,2,3,7,8,9-HxCDF	<0.19		<0.34		106	103
2,3,4,6,7,8-HxCDF	1.3	6.7	1.4	6.2	107	87
1,2,3,4,6,7,8-HpCDF	21	2.4	22	1.3	89	67
1,2,3,4,7,8,9-HpCDF	(0.37)	23	<0.43		85	71
OCDF	<0.55		<1.1		68	67
Non- *ortho*-PCBs						
3,4,4',5-TCB (#81)	1.4	3.6	1.5	7.7	75	72
3,3',4,4'-TCB (#77)	2.9	2.4	2.8	2.6	73	75
3,3',4,4',5-PeCB (#126)	24	6.2	23	1.1	90	93
3,3',4,4',5,5'-HxCB (#169)	36	2.1	35	2.0	94	98

*Mean (n=3)

Parentheses are values beyond LOD under LOQ and values under LOD are show by <LOD.

At-column: Sample solution of 100 μL in final sample solution of 110 μL was injected into HRGC-HRMS. (This is the quantity that it is equivalent to the serum sample of 4.5 g.

Splitless: Sample solution of 2 μL in final sample solution of 20 μL was injected into HRGC- HRMS. (This is the quantity that it is equivalent to the serum sample 2.5 g.)