ANALYSIS OF PCBs IN SERUM BLOOD BY USING GC/NICI-MS

Okimoto M[1]* Enomoto T[1], Kobayashi M[1], Fujii S[1], Jotaki T[2], Nanbu Y[2], Tachino H[2]

[1] JEOL Ltd. 1156 Nakagami-cho, Akishima, TOKYO 196-0022 JAPAN; [2]SRL Ltd. 153 Komiya-cho, Hachiouzi, TOKYO 192-0031 JAPAN

Introduction

In Japan, "Environment and Children's Study (Eco-Chil)" has been carried out since 2011 in response to concerns about effect of chemical compounds on fetal, infant, and childhood growth. Recently, possibility that PCBs effect child growth and cerebral development has been reported and therefore long-term exposure assessment has been needed. However, such fetal or infant samples require rapid and high-sensitive method because of its scarcity and limited amount of sample.

In this study, Negative Ion Chemical Ionization (NICI) method monitors Cl^- ion (*m/z*: 35). Thus, the method is high sensitive analytical method that can selectively detect chlorinated compound. To establish the analytical procedure with limited sample amount, we assayed serum PCBs with NICI and compared it to conventional method, GC-ECD.

Materials and methods

Chemicals and reagents

Kanechlor mixtures (KC-mix) containing equal amounts of KC-300, KC-400, KC-500 and KC-600 were prepared and used as a standard. The PCBs standards were purchased from GL Sciences, Inc. A known amount of 2,2,4,4,5,6-hexabromobiphenyl (IUPAC# 154) was added as the internal standard.

Sample preparation from blood serum

Blood serum (1 g) was saponified with 3 ml of KOH/ethanol solution (1 mol/L) and then extracted with hexane. The extract was washed with deionized water and remaining water are dried with Na_2SO_4 (anhyd.). The washed extart was cleaned-up through Florisil-1% water (w/w) and the elutant was concentrated under nitrogen gas. The internal standard (20 ppb of 2,2,4,4,5,6-hexabromobiphenyl, 50µl) was added to the elutant. The sample was filled up to 0.5 ml with hexane and then injected to GC with the following conditions.

Measurement

GC/NICI-MS method (JMS-Q1000GC MK II : JEOL)

HP-5MS column (30 m × 0.25 mm × 0.25 µm: Agilent Technologies) was used as analytical column. Temperature program was the following condition: from 70°C (2 min) to 185°C at 30°C/min then to 300°C at 6 °C/min (2 min). The other GC conditions were the following: injection volume, 2µl; injector temperature, 300°C; carrier gas, helium; reagent gas, methane (99.999%). Two selected ions, m/z = 35 and 81, were monitored for Cl^- and Br^- respectively

Packed Column GC-ECD method (GC-17A : Shimadzu)

Chromosorb W 80/100 AW-DMCS (30 m × 2.6 mm: GL Sciences) was used as analytical column. GC conditions were the following: oven temperature, 210°C; injection volume, 5µl; injector temperature, 250°C; carrier gas, helium; detector temperature, 310°C; make-up gas, nitrogen.

The calculations of PCBs concentration are performed using CB_0 (%) of each peak.

Results and discussion

GC/NICI-MS Calibration curve

Calibration curve of GC/NICI-MS was shown in Fig.1.

The linearity of calibration curve in a range from 1.5 to 100 ppb gives $R_2 > 0.999$ and the rate of deviation is within 10% .

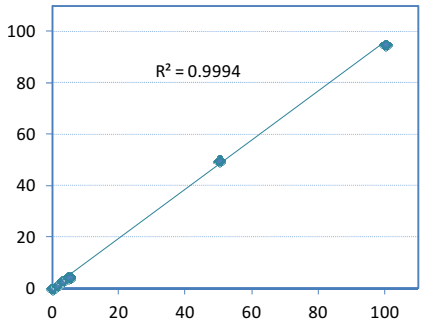

Fig. 1 GC/NICI-MS calibration curve. (ppb)

Correlation between GC/NICI-MS and Packed Column GC-ECD

The PCBs concentrations in the blood serum samples are shown Table.1 (n=39), and correlation between GC/NICI-MS and Packed Column GC-ECD are shown Fig.1.

Concentration of blood serum samples in this study has a range from 0.1 to 1 ppb. The difference in quantitative value between GC/NICI-MS and packed column GC/ECD is within 20%. The coefficient of determination is $R_2 > 0.98$. Therefore, we confirmed GC/NICI-MS have an enough correlation with conventional method.

Table 1. PCBs concentrations in the blood serum sample (ng/g).

	NICI-MS	GC-ECD	NICI/ECD(%)		NICI-MS	GC-ECD	NICI/ECD(%)
No.1	0.84	0.86	98.0	No.21	0.17	0.2	85.6
No.2	0.63	0.7	90.3	No.22	1.01	1.1	91.9
No.3	0.64	0.65	97.7	No.23	0.82	0.89	92.4
No.4	0.96	0.99	96.8	No.24	0.32	0.36	89.7
No.5	0.38	0.45	85.5	No.25	0.2	0.24	83.6
No.6	0.4	0.44	90.4	No.26	0.59	0.65	90.5
No.7	0.41	0.46	89.8	No.27	0.15	0.19	80.6
No.8	0.57	0.59	96.7	No.28	0.33	0.4	83.1
No.9	0.18	0.24	76.1	No.29	0.3	0.38	80.2
No.10	0.32	0.37	86.3	No.30	0.37	0.44	84.8
No.11	0.33	0.4	82.9	No.31	0.3	0.36	83.2
No.12	0.84	0.84	100.4	No.32	0.41	0.49	83.9
No.13	0.2	0.23	86.5	No.33	0.62	0.71	87.8
No.14	0.4	0.46	86.3	No.34	0.75	0.87	86.4
No.15	0.84	0.9	93.4	No.35	0.31	0.39	80.6
No.16	1	0.99	100.6	No.36	0.38	0.48	80
No.17	1.2	1.2	100.1	No.37	0.65	0.75	86.7
No.18	0.35	0.35	99.9	No.38	0.42	0.51	81.8
No.19	0.16	0.19	83.6	No.39	0.4	0.47	85.4
No.20	0.15	0.18	81.6				

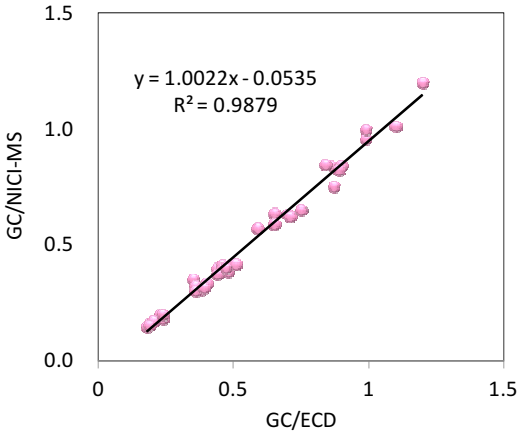

Fig 1. Correlation between GC/NICI-MS and Packed Column GC-ECD. (ng/g)

Chromatogram of blood serum sample

The mass chromatogram of GC/NICI-MS is shown in Fig.2 and the chromatogram of packed column GC-ECD is shown in Fig.3 (m/z = 35). In both methods, peaks of PCBs are detected after *p,p'*-DDE.

In NICI method, individual isomers are observed due to separation in capillary column. Major peaks of these isomers, which have been reported mainly in human blood, are IUPAC #153 (2,2',4,4',5,5'-HexaCB), #138 (2,2',3,4,4',5'-HexaCB), and #180 (2,2',3,4,4',5,5'-HeptaCB). Calculated result of the method detection limit (MDL) in NICI method is 0.02ppb.

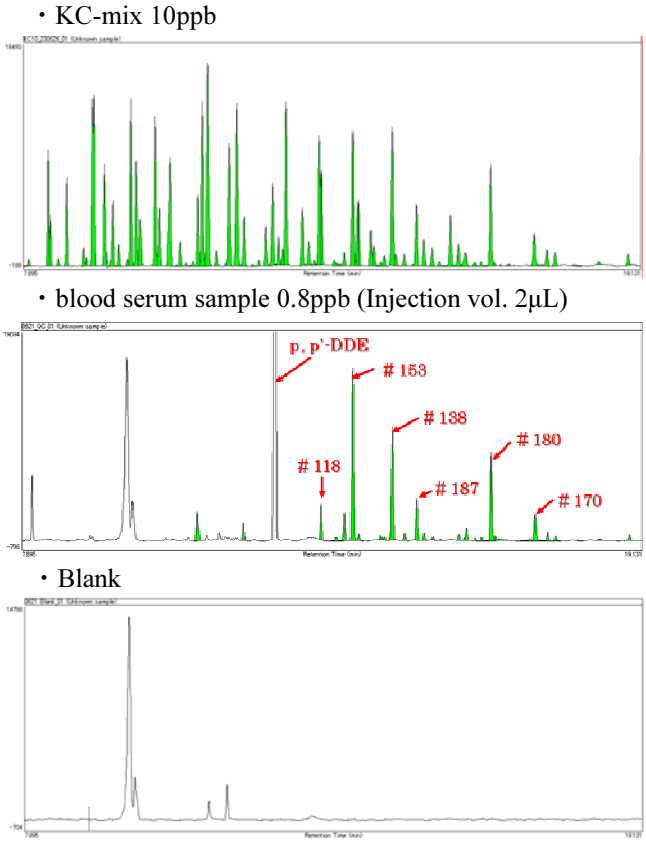

Fig. 3. Mass Chromatogram(m/z = 35) of GC/NICI-MS.

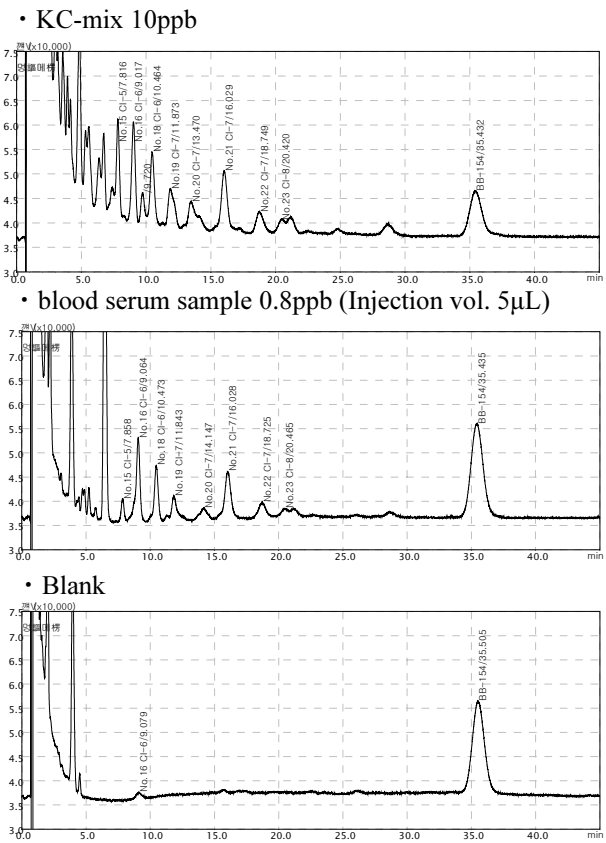

Fig. 2. Chromatogram of Packed Column GC-ECD.

These results in this study show GC/NICI-MS have an enough correlation with GC/ECD, which is conventional method, in analysis of PCBs in blood serum sample. Additionally, the MDL of the NICI (0.02ppb) is one-fifth MDL of the ECD (0.11ppb). Thus, NICI allows high sensitivity method for PCBs analysis.

These results allow downsizing of pretreatment by reduction of sample and simplified pretreatment. We are planning to establish the analysis method using a few hundred of blood sample.

References

1. Jacobson JL, Jacobson SW. (2003); *Jpediatr,* 143: 780-8
2. Reiko N, Yuki A, Tsuguhide H, and Takao I. (2003); *Reprinted from FUKUOKA ACTA MEDICA,* 94(5): 144-147
3. Reiko N, Matayoshi N, Hironori H, Tsuguhide H, and Takao I. (1999); *Reprinted from FUKUOKA ACTA MEDICA,* 90(5): 184-191