ALTERATIONS OF THE BACKGROUND DIOXINS LEVELS IN HUMAN SERUM OF GENERAL POPULATION IN JAPAN

Osamu Aozasa, Ayumi Mochizuki, Teruyuki Nakao, Souichi Ohta, Hideaki Miyata and Yoshinori Fujimine[1]

Faculty of Phermaceutical Scienceis, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka, 573-0101, Japan ; Otsuka Assay Laboratories Otsuka Pharmaceutical Co., Ltd., 224-18, Aza Ebisuno Hiraishi, Kawauchi-cho, Tokushima, 771-0195, Japan[1]

Introduction

Internationally, human blood is used as a typical sample for extensive investigations into human pollution by dioxins (PCDDs, PCDFs, mono- and non-ortho Co-PCBs.), because blood samples are available from all members of the general population compared to breast milk and adipose tissue in human anatomy. In most of cases, the degree of the human exposure is assessed based on the their concentrations in blood. It is important to examine in detail alterations in the background level of dioxins in blood by daily life factors in order to identify human body pollution due to high-density exposure. However, the current analysis of dioxins in blood requires the time-consuming sample clean up involving the extraction and complicated purification before quantification can be made by HRGC-HRMS due to the trace levels of these concentrations and limited sample volume. The complicated purification method needs a large volume of organic solvent and a prolonged sample handling time. In this study, the complicated purification process composed of repeating open column chromatography twice was simplified by appraising the useful components and a sufficient amount of adsorbents contained in the column. Alterations in the background level in the general Japanese population were clarified by monitoring the dioxin concentrations in human blood for 3 months.

Materials and Methods

The 25 blood samples were taken from healthy 5 fasting donors 5 times over 3 months between April and June 2000. Donor ages ranged from 21 to 23-years old. The blood samples were analyzed for 7 isomers of 2,3,7,8-PCDDs, 10 of 2,3,7,8-PCDFs, 8 of mono- and 4 of non-ortho Co-PCBs. Twenty five grams of serum prepared from whole blood was spiked with a mixture of ^{13}C-labeled internal standards for all 17 2,3,7,8-isomers and 10 Co-PCBs (each 50 pg). The sample was prepared by mechanical shaking at room temperature for 2 hours with 2N KOH solution. The solution was extracted 2 times with 30 mL of hexane. The hexane phase concentrated to 10 mL was passed through a column containing 1g of the silver nitrate coated silica gel and eluted with 10 mL of hexane. The eluted solution was loaded to an active carbon-dispersed silica gel column (0.1g), and separated into two fractions. The first fraction containing mono-ortho Co-PCBs were eluted with 5 mL of hexane and 25% methylene chloride in hexane. PCDDs, PCDFs and none-ortho Co-PCBs were eluted with 30 mL of toluene as the second fraction. These eluates to be spiked recovery standards were respectively concentrated to 10 μL of n-nonane. Determinations were performed with HP 6890 gas chromatograph and JEOL JMS 700M mass spectrometer at resolution 10000 by EI-SIM mode. Lipid concentration was determined gravimetrically using 10g of serum.

Results and Discussion

The complicated current purification procedure of the current analysis of dioxins for human blood was simplified to perform high quality and high throughput measurements for human blood. The current procedure consists of two open column techniques using a multi layer silica gel column and an active carbon-dispersed silica gel column as shown in Fig 2. Fig 1 shows the total ion chromatogram of the HRGC/LRMS analysis in a human serum pool sample extract after purification by each silica gel constituting the multi layer column. The peaks of the matrix including in blood serum extract without purification were remarkably decreased by purification using multi layer silica gel column (A and B in Fig. 1). The reduction of purification efficiencies is observed when H_2SO_4/silica and KOH/silica were independently used in a open column (C, D and E). However, the chromatogram of $AgNO_3$/silica resembles that of the multi layer column (F and G). These results have clearly shown, that a purification procedure, using only 1g of 10% $AgNO_3$/silica gel, facilitates clean-up by removing the matrix of blood serum equivalent to that of multi layer silica gel column. In addition, a sufficient amount of active carbon dispersed silica gel was examined. The findings indicated that it is possible for 0.1g of carbon silica gel, equivalent to 1/10 the weight used in the current analysis, separates and recovers the dioxins in purified extract of blood serum. (data not shown) As shown in fig. 2, which summarizes above results, the simplified method cut about 90% of the absorbents and solvent used compared to that used by the current procedure. Curtailment of reagents reduces handing time and cost, and helps to minimize interference problems. This optimized clean-up technique was used for the subsequent investigation of alterations in the background level in the general population in Japan.

Table 1 shows the total TEQ concentrations of PCDDs, PCDFs and Co-PCBs in blood serum from 5 healthy donors collected 5 times over 3 months (pg TEQ/g lipid). In the 5 donors, there was no significant alteration of the total TEQ concentrations observed, showing a coefficient variation (C.V.) of total TEQ concentrations in the range of 12% to 44% for PCDDs, 8.6% to 29% for PCDFs and 9.2% to 27% for Co-PCBs. In addition, the total TEQ levels of PCDDs of the blood serum in all 5 donors were higher than those of PCDFs throughout the 3-month monitoring period. Compared to PCDDs and PCDFs, Co-PCBs values differ among donors. However, in each donor, there was no significant change in the serum concentration ratios of the three dioxins throughout the monitoring period. From these results, the dioxins concentrations in human blood were confirmed to have remained at a tolerable level for 3 months. Therefore, it was concluded that blood serum was available as an indicator sample for detection of a remarkable elevation of dioxins levels considered to be indicate human body pollution due to high-density exposure.
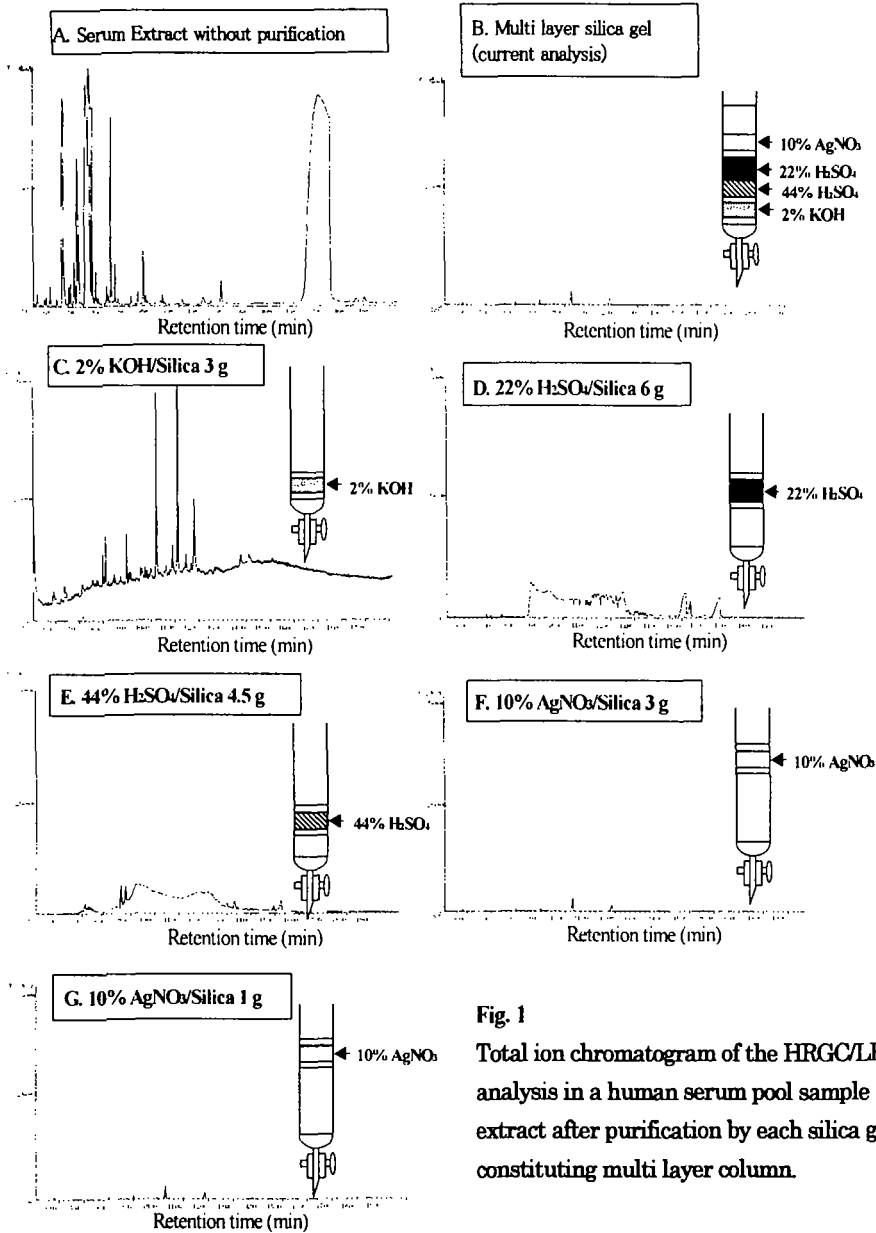
Fig. 1

Total ion chromatogram of the HRGC/LRMS analysis in a human serum pool sample extract after purification by each silica gels constituting multi layer column.
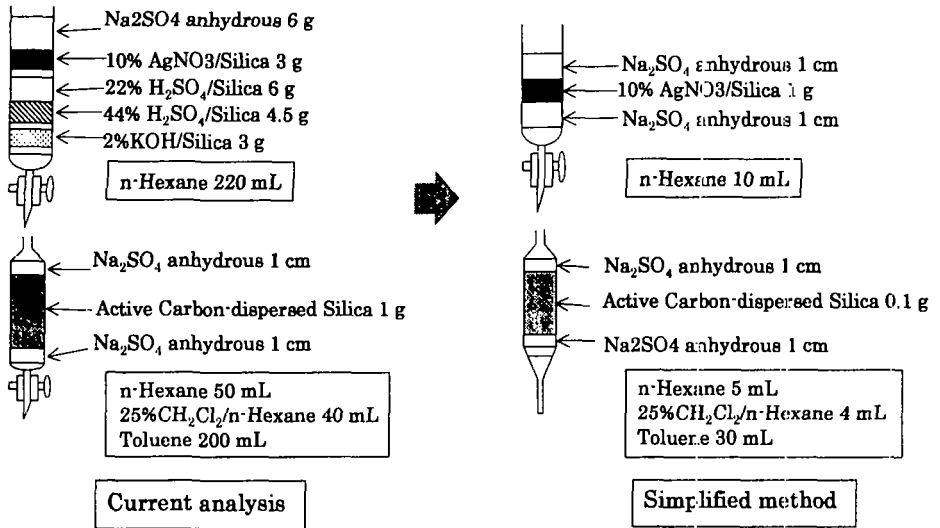
Fig. 2 Reduction of absorbents and solvents by simplified method

Table 1 Total TEQ concentrations of PCDDs, PCDFs and Co-PCBs in blood serum
from 5 healthy donors collected 5 times over 3 months (pg TEQ/g lipid)

Donor	Dioxins	Cocentration (pg-TEQ/g lipid)						
		1 st	2 nd	3 rd	4 th	5 th	average	C.V.(%)
No.1	PCDDs	9.1	6.3	11	6.2	12	8.9	30
	PCDFs	7.1	5.3	4.8	5.0	9.0	6.2	29
	Co-PCBs	7.5	4.1	6.5	4.7	7.9	6.1	27
	Total	24	16	22	16	29	21	26
No.2	PCDDs	6.1	2.6	7.7	11	9.0	7.3	44
	PCDFs	4.6	4.6	6.0	6.4	5.4	5.4	15
	Co-PCBs	3.4	3.9	3.8	5.1	4.4	4.1	16
	Total	14	11	18	22	19	17	26
No.3	PCDDs	10	11	10	12	13	11	12
	PCDFs	7.0	6.8	8.4	7.2	7.2	7.3	8.6
	Co-PCBs	8.1	7.2	7.5	9.6	9.5	8.4	13
	Total	25	25	26	29	30	27	8.7
No.4	PCDDs	12	14	13	13	19	14	20
	PCDFs	5.1	5.5	7.4	6.8	7.4	6.4	17
	Co-PCBs	8.3	9.6	11	10	11	10	11
	Total	25	29	31	30	37	30	14
No.5	PCDDs	13	8.9	15	22	19	16	33
	PCDFs	7.4	7.7	9.0	12	12	9.6	23
	Co-PCBs	15	13	16	14	13	14	9.2
	Total	36	30	40	48	44	40	18