Rapid clean-up method for the determination of PCDDs, PCDFs and coplanar PCBs in human milk samples

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INTRODUCTION

Although mother's breast milk is very important foodstuff for newborn babies, it has been identified that mother's milk is one of the major channels of organochlorine contaminants. Since Rappe and coworkers reported the levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in human milk samples[1], many studies have been made about the levels of PCDDs and PCDFs in mother's milk samples[2].

In addition to PCDDs and PCDFs, coplanar polychlorinated biphenyls (PCBs) which have similar toxicity to dioxins were found in human milk[3],[4]. Those studies show the levels of coplanar PCBs are much higher than the levels of PCDDs or PCDFs[5].

Determination of PCDDs, PCDFs and coplanar PCBs in human milk samples is very difficult, because the levels of these compounds are extremely low, the amount of sample are limited and in most cases high sensitivity and selectivity are required for an analytical method.

In this paper, we report a clean-up method which consist of three different column systems and the performance of this method.

EXPERIMENTAL

Materials

All solvents were residual pesticide analytical reagent grade (Wako chemical). Potassium oxalate, sodium sulfate, potassium hydroxide, sulfuric acid and silver nitrate were analytical reagent grade. All reagents were used without further purification.

Silica gel (Merck, Kieselgel 60) was washed with methanol and activated at 130 °C for over night before use. Activated carbon-impregnated silica gel was purchased from Wako chemical, which was sold as dioxin analytical reagent grade. Sep-Pak plus cartridge column (Waters) was used for neutral alumina column clean-up.

For internal standards, ^{13}C-labeled 2,3,7,8-substituted PCDDs, PCDFs and non-*ortho* substituted PCBs (Cambridge Isotope Lab.) were used.

Method

The schematic view of this method is shown in Fig. 1.

Extraction

Commercial cow's milk (50 g) was fortified with 1-2 ng of ^{13}C - labeled compounds, and then subjected to extraction with 0.5 g of potassium oxalate, 50 ml of ethanol, 25 ml of diethyl ether and 25 ml of hexane. Milk sample was extracted more two times with 25 ml of hexane. Hexane layer was combined and concentrated to dryness by rotary evaporator. Fat content was determined by measuring the residue.

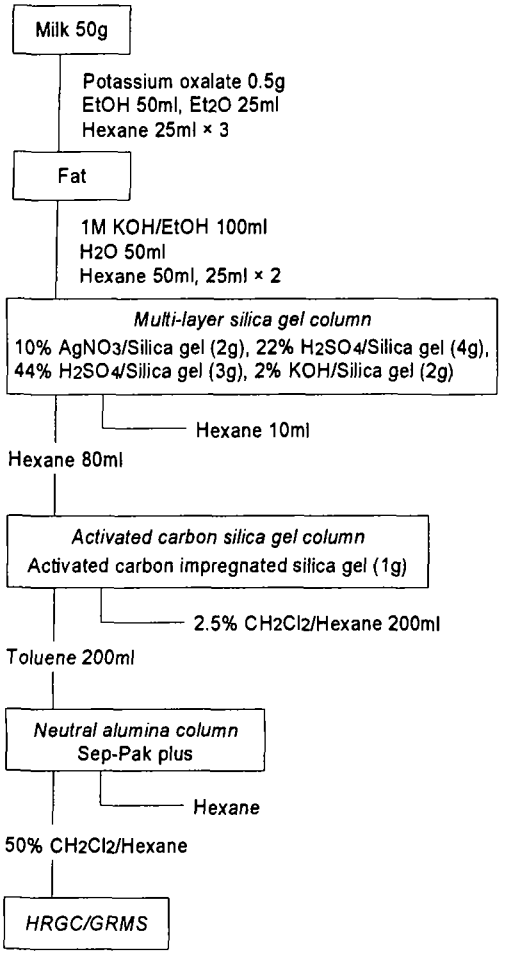

Fig. 1 The Scheme of the analytical procedure

Fat was then saponified with 100 ml of 2 mol/l of potassium hydroxide in ethanol. Digested sample was diluted with 50 ml of water, and then extracted with hexane. Hexane layer was washed with 2 % aqueous sodium chloride, and then dried with anhydrous sodium sulfate.
The extract was concentrated to ca. 10 ml.

Multi-layer silica gel column chromatography
To remove fatty acid and other matrices, multi-layer silica gel column was used. The extract was applied on this column, and eluted with hexane. The first eluate of 10 ml of hexane was discarded, and then the subsequent eluate of 80 ml of hexane was collected.
The eluate was concentrated to ca. 0.3 ml.

Activated carbon column chromatography
PCDDs, PCDFs and coplanar PCBs were separate from other organochlorine compounds by activated carbon column. Activated carbon impregnated silica gel (1 g) was packed in a glass column (200 × 10 mm i.d.). The eluate from silica gel column was transferred to this column. The first eluate of 200 ml of 2.5 % dichloromethane in hexane was discarded. Then the subsequent eluate of 200 ml of toluenewas collected in which fraction PCDDs, PCDFs and coplanar PCBs appeared.The toluene fraction was concentrated to dryness, and the residue was dissolved in 0.5 ml of hexane.

Neutral alumina column chromatography
Toluene fraction from previous column was transferred to Sep-Pak plus Alumina-N cartridge column. The first eluate of hexane was discarded. The second eluate of 30 ml of 50 % of dichloromethane in hexane contained PCDDs, PCDFs and coplanar PCBs. The second fraction was concentrated to evaporate. The residue was dissolved in 20 µl of toluene, and then analyzed by HRGC/HRMS.

HRGC/HRMS Determination
Capillary column gas chromatograph/high resolution mass spectrometer (HRGC/HRMS) was used for the determination of dioxins and PCBs. HP 5890A (Hewlett-Packard) gas chromatograph equipped with a splitless injector was used. GC column was PTE-5 (Supelco, 30m × 0.25 mm i.d., 0,25µm film thickness). Oven temperature was as follows; 120 °C (1 min.) - 20 ° C/min. - 260 °C (10 min.).
JMS-SX102 (JEOL) mass spectrometer was operated in selected ion monitoring (SIM) mode. The resolution of MS was over 9,000. Two ions were monitored for each congener.

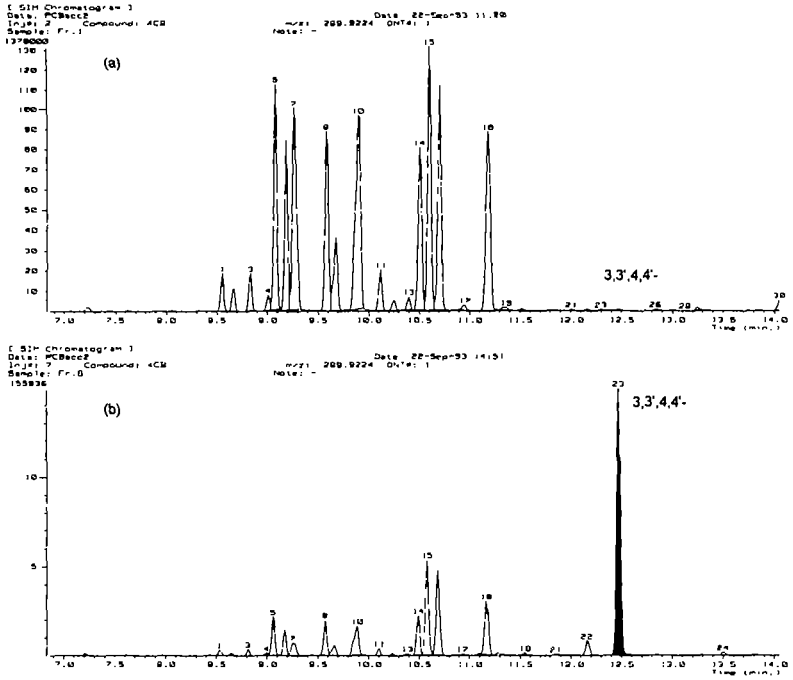

Fig.2 SIM chromatograms of $^{12}C_{12}$-tetrachlorobiphenyls
(a) without activated carbon column clean-up
(b) with activated carbon column clean-up

RESULTS AND DISCUSSION

Because of the limitation of milk sample volume, it is desirable that determination of fat content and extraction of dioxins and other contaminants can be achieved together. We have adopted two step extraction[6]. In the first extraction, milk was denatured with potassium oxalate and ethanol and extracted with diethyl ether and hexane. The extract was evaporated to dryness and measured for calculating of fat content. The extracted fat contained dioxins and other organochlorine compounds. The whole of fat was saponified with potassium hydroxide in ethanol at room temperature. Then dioxins and other compounds were extracted with hexane.

To remove fatty acid and other matrices, liquid-liquid partitioning methods, such as sulfuric acid treatment, are often used. Liquid-liquid partitioning can be replaced by H_2SO_4/Silica gel partitioning. We have chosen multi-layer silica gel column developed by Miyata et al.[7]. In elution study, the first hexane fraction 10 ml didn't contain dioxins and coplanar PCBs and so this fraction was abandoned. dioxins and coplanar PCBs. Dioxins and coplanar PCBs were recovered in the fraction of 10-80 ml.

After multi layer silica gel column separation, the sample was subjected to activated carbon column chromatography to remove many isomers of PCBs existing in milk and other biological samples. Since activated carbon show high affinity for planar compounds such as dioxins, activated carbon column chromatography is one of the most effective clean-up procedures for analysis of PCDDs and PCDFs[8]. We have used this clean-up procedure for separation PCDDs, PCDFs and coplanar PCBs from other PCBs or other organochlorine pesticides. When 5 % of dichloromethane in hexane was used as first eluent, a significant amount of 3,3',4,4'-tetrachlorobipheny was eluted in this fraction. This problem has been solved by means of reducing the proportion of dichloromethane in first eluent. By using

reduced proportion of dichloromethane in first eluent (2.5 %), the loss of PCDDs, PCDFs and coplanar PCBs in the dichloromethane/hexane fraction was stopped and they were recovered in toluene fraction. Highly chlorinated congeners show the tendency to be absorbed on activated carbon column and therefore 200 ml of toluene was needed to recover almost all of the spiked dioxins and coplanar PCBs. SIM chromatograms of T_4CBs are shown in Fig. 2. Big peaks from some isomers of T_4CBs observed (Fig. 2(a)) in the milk extract, but these peaks were removed by activated carbon column clean-up (Fig. 2(b)).

To remove the non-coplanar isomers of PCBs which were not separated with activated carbon column completely, neutral alumina column was used. We used Sep-pak cartridge column to simplify this procedure. Non-coplanar isomers of PCBs were eluted in hexane, and dioxins and coplanar PCBs were recovered in 50 % of dichloromethane in hexane.

The overall recoveries of fortified compounds are listed in Table 1. The recoveries of O_8CDD, O_8CDF, T_4CB and P_5CB are relatively low. The strong affinity of octachlorinated compounds to activated carbon may decrease the recovery of these compounds and weak affinity of lower chlorinated biphenyls may cause the elution of these congeners in the first eluent.

PCDDs	Recovery(%)	PCDFs	Recovery(%)	coplanar PCBs	Recovery(%)
T_4CDD	87	T_4CDF	84	T_4CB	64
P_5CDD	96	P_5CDF	88	P_5CB	69
H_6CDD	89	H_6CDF	88	H_6CB	81
H_7CDD	83	H_7CDF	82		
O_8CDD	70	O_8CDF	67		

Table 1. Recoveries of ^{13}C-labeled compounds fortified to milk samples.

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