SIMPLE SOLID-PHASE LIPID EXTRACTION OF DIOXINS FROM MATERNAL BREAST MILK.

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Introduction

A method for solid-phase extraction (SPE) of lipids containing polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and co-planer polychlorinated biphenyls (Co-PCBs) from maternal breast milk has been developed. Maternal breast milk samples were extracted by 100 ml of diethyl ether through the cleaned kieselguhr filled glass columns. After clean-up, the extracts were analyzed by the high-resolution gas chlomatography / high-resolution mass spectrometry (HRGC/HRMS) [1]. Quantification has been done using internal standards of $^{13}C_{12}$ labeled PCDDs, PCDFs and Co-PCBs and calibration curves based upon five concentration levels. The integrated SPE method for maternal breast milk was fast, less laborious than the methods using liquid-liquid extraction and contributed to lower consumption of organic solvents.

Methods

Samples

Pooled samples of maternal breast milk were studied. The samples were obtained from volunteers at the Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan. Samples were stored at –20℃ until analysis.

Sample extraction and clean-up

Lipid extraction

Liquid-Liquid extraction

Maternal breast milk was extracted using a quantitative liquid-liquid extraction method as a standard method of analysis by most hygienic chemists. Potassium oxalate (0.25 g) and ethanol (25 ml) were added to 10ml maternal breast milk. The lipid was isolated by liquid-liquid extraction with diethyl ether (50 ml) and petroleum ether (50 ml). The lipid-containing ether layer was dehydrated by sodium sulfate and evaporated to dryness. The weight of lipid was measured by a electric balance.

SPE

The SPE column consisted of 10 g wide-pore kieselguhr was used for the lipid extraction from maternal breast milk. Ten ml of maternal breast milk was applied to the SPE column for 20 min, and the lipid was eluted with 100 ml of diethyl ether at a flow-rate of 2.5 ml/min. The lipid-containing ether layer was evaporated to dryness, and weight of lipid was determined.

Multi-layer column chromatography for clean up

Extracted lipid was dissolved in 3ml of n-hexane. 40 pg each of the internal standards ($^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-Co-PCBs) were added to the solution. The solution was cleaned up on a multi-layer column containing Na_2SO_4 (1.5g), silica (0.9g), $AgNO_3$-silica (3g), silica (0.9g). 22%(W/W) H_2SO_4-silica (6g), 44%(W/W) H_2SO_4-silica (4.5g), silica (0.9g), 2%(W/W) KOH-silica (3g), silica (0.9g) and Na_2SO_4 (1.5g). Before loading the sample, the column was washed with 100 ml of n-hexane. After the application of the sample, dioxin fraction was eluted by 120 ml of n-hexane at the flow-rate of 2.5 ml/min. After addition of about 20 μ l of n-decane, the elute was concentrated at 50℃ on a rotary evaporator to a volume of about 2 ml.

Active carbon-impregnated silicagel column chromatography for clean-up

The previous solution was applied to the active carbon-impregnated silicagel column. The column packed Na_2SO_4 (3g), active carbon-impregnated silicagel (0.5g) and Na_2SO_4 (3g). The column was washed 20 ml of with n-hexane before loading the sample. Mono-ortho-PCBs were eluted by 100 ml of n-hexane-dichloromethane (4:1 V/V) at the flow-rate of 2.5 ml/min. PCDDs, PCDFs and non-ortho-PCBs were then eluted by 100 ml of toluene at the flow-rate of 2.5 ml/min.

Preparation of the sample for HRGC/HRMS.

PCDDs, PCDFs and non-ortho-PCBs fraction was evaporated on a rotary evaporator to 0.5 ml and transferred to a GC vial tube. The remaining solvent was evaporated under a gentle stream of nitrogen. The walls of the flask were flushed with 10 to 20 μ l volumes of n-hexane.

A 1.5 μ l sample was injected to HRGC/HRMS and analyzed for PCDDs and PCDFs. After the analysis of PCDDs and PCDFs, mono-ortho-PCBs fraction was evaporated on a rotary evaporator to 0.5 ml and transferred to the PCDDs, PCDFs analyzed GC vial tube. The remaining solvent was evaporated under a gentle stream of nitrogen. The walls of the flask were flushed with small volumes of n-hexane, and concentrated to 10 to 20 μ l. The 1.0 μ l sample was injected to HRGC/HRMS for analysis of Co-PCBs. Finally, the values of 2,3,7,8-TCDD Toxicity Equivalence Factor (WHO-TEFs)[2] was used for calculation of TEQ.

Results and Discussion

Table 1 shows extracted lipid amounts from pooled maternal breast milk by both SPE method and liquid-liquid extraction method. The reproducibility was also examined. The amount of extracted lipid by the SPE method (Avg.=2.73%) was almost the same with liquid-liquid extraction method (Avg.=2.78%) . The reproducibility with the SPE method was good (C.V.=4.6%).

Table 1. Reproducibility Test of Lipid-Extraction from Maternal Breast Milk

	Extracted Lipid (%)				Avg.	S.D.	C.V.(%)
	1st	2nd	3rd	4th			
Solid-Phase extraction	2.90	2.63	2.76	2.65	2.73	0.125	4.6
Liquid-Liquid extraction	3.03	2.82	2.70	2.60	2.78	0.184	6.6

The 10ml of the amounts of maternal breast milk was used.

The analytical concentrations for the total PCDDs/PCDFs and total Co-PCBs are presented in Table 2. When the results are expressed in pg TEQ per gram of fat, the reproducibility was good (C.V. =0.33～5.78%). The mean results (Total PCDFs+PCDDs+Co-PCBs) of SPE method was 17.5 pgTEQ/g-lipid with C.V.=0.33% (n=3). The mean concentration (Total PCDFs+ PCDDs+Co-PCBs) by the liquid-liquid extraction method was 17.9 pgTEQ/g-lipid with C.V.=0.46% (n=4). The same results as Total TEQ values were obtained between the analysis of the SPE method and liquid-liquid extraction method.

Table 2. Quantitative Variance of Dioxins and Coplanar PCBs Extracted Maternal Breast Milk (10ml)

a) Solid-Phase Extraction

(pg-TEQ/g-lipid)	Trial1	Trial2	Trial3	Avg.	S.D.	C.V.(%)
Total PCDFs	4.95	4.55	4.44	4.65	0.27	5.78
Total PCDDs	6.58	6.93	6.88	6.80	0.19	2.79
Total Coplanar PCBs	6.06	6.04	6.17	6.09	0.07	1.15
Total (PCDFs+PCDDs+Coplanar PCBs)	17.6	17.5	17.5	17.5	0.06	0.33

b) Liquid-Liquid Extraction

(pg-TEQ/g-lipid)	Trial1	Trial2	Trial3	Trial4	Avg.	S.D.	C.V.(%)
Total PCDFs	4.63	4.88	5.08	5.07	4.92	0.21	4.30
Total PCDDs	6.78	6.48	6.38	6.27	6.48	0.22	3.38
Total Coplanar PCBs	6.59	6.56	6.48	6.49	6.53	0.05	0.82
Total (PCDFs+PCDDs+Coplanar PCBs)	18.0	17.9	17.9	17.8	17.9	0.08	0.46

Conclusion

SPE with wide-pore kieselguhr column was successfully applied for the determination of dioxins from maternal breast milk. SPE was preferred to liquid-liquid extraction techniques for its smaller solvent volumes and easy handling. The SPE method allowed for further reduction of solvent volumes (compared with liquid-liquid extraction). This method was considered to be one of ideal methods for ultratrace determinations. The risk for sample contamination was decreased by using this method.

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Reference

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