DETERMINATION OF PERSISTENT ORGANOCHLORINE PESTICIDES IN HUMAN BREAST MILK BY SOLID PHASE EXTRACTION AND GC/HRMS

Seo Jungju[1], Chang Yoon-Seok[2] , Shin Jeong-Hwa[1] ,Choi Jong-Woo[3]
[1]Korea Basic Science Institute, Seoul, 136-701 Korea; [2]POSTECH, Pohang, 790-784 Korea, [3]National Institute of Environment Research, Incheon, 404-708, Korea

Abstract
A method for the analysis of organochlorine pesticides (18 species) in human breast milk by solid phase extraction and gas chromatography-high resolution mass is described. Organochlorine pesticides in breast milk samples are extracted by C18-NH2 solid phase extraction and purified by silica-Florisil column. Since the extraction and cleanup steps for biota samples generally are more complicate than those for water or sediment samples, thus we compared with solid phase extraction state of each sample work-up step. 2mL of breast sample was necessary to obtain sub ng sensitivity. By applying this developed method, their overall recoveries ranged between 81 – 109 % in breast milk sample which was added to standard sample. For organochlorine pesticides used in this study, the quantitative accuracy, elution pattern on each SPE columns, and detection limits were also investigated. To evaluate the developed method, comparison with GC-MS and interlaboratory analysis were performed.

Introduction
Persistent organic pollutants (POPs) have spread throughout the global environment to threaten human health and damage ecosystems, with evidence of POPs contamination in wildlife, human blood, and breast milk documented worldwide[1]. Since 1976 the World Health Organization through its GEMS/Food Programme has collected and evaluated information on levels of persistent organic pollutants in foods, including human milk[2]. To provide comparable world wide data for human breast milk, UNEP suggested guide line of sampling and analysis[3].
The most difficult point in the human breast milk analysis is limited sample amount of biological sample to analysis the whole POPs. The conventional approach to determine persistent organochlorines in human breast milk involves liquid-liquid extraction, followed by a multi-step purification using various sorbents using GC-MS or GC-ECD[4-7]. Analysis by HRMS to enhance sensitivity and effective preparation methods to eliminate interference can be an alternative. Limited 13C labelled standards of organochlorine pesticides and high necessary sample amounts were most difficulty problem to adept HRMS in human breast milk.
In this study, simple SPE extraction and clean-up to quantify persistent organochlorines in high lipid containing breast milk sample by GC-HRMS. Two instrumental methods and interlaboratory study were compared to investigate the effectiveness of extraction for analysis of organochlorine pesticides.

Materials and Methods
Human breast milk samples (n = 20) were collected from mothers in urban area of Seoul, the capital of Korea. We obtained informed consent from all the mothers donated milk samples. Samples were collected in chemically cleaned containers and stored at -20°C until analysis. Each two sample were pooled and divided again individual two homogeneous samples to the interlaboratory study.
Chemicals analyzed in this study were DDTs (p,p'-DDE, p,p'-DDT, and p,p'-DDD), HCHs (α-, β-, and γ-isomers), HCB, Heptachlor, Aldrin, Heptachlor Expoxide, Dieldrin, chlordane compounds (CHLs: trans-nonachlor, cis-nonachlor, transchlordane, cis-chlordane, oxychlordane), Endrin, and Mirex. C13 labelled standards were purchased from Cambridge Isotope Laboratory.
Approximately 2 g of human breast milk sample was added to 2mL formic acid and 2mL deionized water. After spike 13C labelled standard, sampled was introduced activated C18-NH_2 cartridge. On the SPE vacuum manifold, stack a sodium sulfate SPE cartridge below each C18 cartridge. Elution of all organochlorine pesticides including toxaphene was carried out within 20 ml hexane. Further cleanup was carried by silica and Florisil SPE cartridge on the vacuum. The identification and quantification were performed using a gas chromatograph (GC: Agilent 6890 series) with an auto-injection system and high resolution MS (JEOL JMS-700D) with a resolving power of more than 10,000. Recoveries for the 13C12-labeled OCPs were within 50-120%.

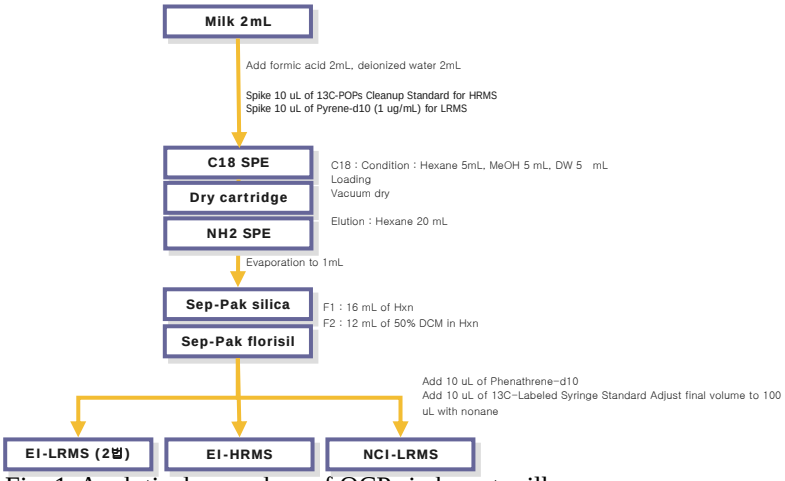

Fig. 1. Analytical procedure of OCPs in breast milk.

Results and Discussion

Both the selection of solvent and the extraction method can be critical in obtaining a satisfactory recovery and sensitivity of the organochlorine pesticides from breast milk. To use of small amounts (2mL) of breast milk sample, solid phase extraction was tested. Extraction and dilapidation ware carried simultaneous by C18-NH2 cartridge. All analytes were completely eluted within 20 ml hexane 100% eluent on C18-NH2-SPE with 85-111 % of recoveries.

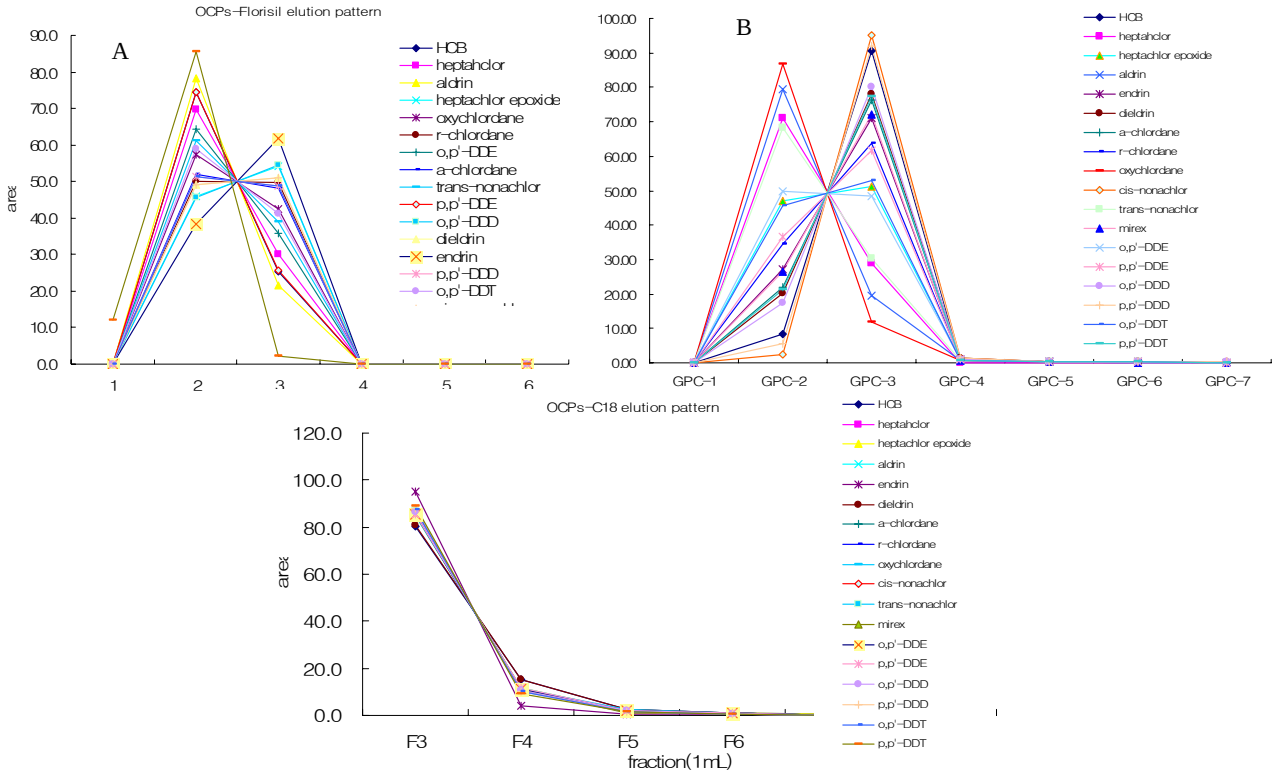

Fig 2 Elution patterns of organochlorine pesticides on A: Florisil-SPE with 15% Ethylacetate in Hx 5mL, B: BioBeads SX-3 GPC column with hexane:dichloromethane(1:1/v:v)each 70 mL, C: C18-SPE with Hexane

Table 1 SPE recoveries of organochlorine pesticides in various sorbents.

Compounds	C18+NH2	florisil+silica	GPC
Aldrin	85.5	74.7	104.5
Dieldrin	94.5	83.2	83.1
Endrin	92.8	86.5	91.5
α-Chlordane	94.5	88.1	80.2
γ-Chlordane	94.5	92.5	80.8
Oxychlordane	93.0	90.6	79.5
Nonachlor-trans	91.3	85.0	77.8
Nonachlor-cis	91.4	83.1	75.4
o,p'-DDD	95.1	87.3	82.1
p,p'-DDD	86.4	84.0	82.8
o,p'-DDE	93.2	84.7	88.1
p,p'-DDE	95.1	93.5	93.1
o,p'-DDT	93.6	89.8	87.3
p,p'-DDT	97.0	95.0	96.5
Heptachlor	86.8	87.6	77.1
Heptachlor epoxide	90.3	87.4	77.4
Hexachlorobenzene	103.7	89.5	87.5
Mirex	110.9	95.5	98.0

The isotope-labelled internal standards and organochlorine pesticides standards were spiked into the control milk, and then extracted, purified, and analyzed by the proposed method. The estimated detection limits (EDLs) and analyte recoveries spiked milk were listed in Table 2. Recoveries were 81–109% with RSD of 1–13%. The estimated detection limit of all organochlorine pesticides in the breast milk tested were 0.01–1.12ng/g (S/N>2.5).

Table 2 EDLs and recoveries of organochlorine pesticides from spiked breast milk (n=5)

Compounds	Recovery (%)	RSD (%)	Estimated Detection Limit (ng/g)
Aldrin	81.4	1.1	0.03
Dieldrin	91.1	2.3	0.05
Endrin	90.6	5.6	0.18
α-Chlordane	85.7	2.2	0.01
γ-Chlordane	108.6	7.0	0.01
Oxychlordane	98.8	6.5	0.10
Nonachlor-trans	84.9	4.8	0.04
Nonachlor-cis	86.3	5.0	0.10
o,p'-DDD	101.5	7.5	0.02
p,p'-DDD	83.3	6.4	0.10
o,p'-DDE	87.4	7.9	1.12
p,p'-DDE	81.2	5.5	0.18
o,p'-DDT	81.4	3.8	0.02
p,p'-DDT	83.5	4.9	0.22
Heptachlor	81.2	5.2	0.77
Heptachlor epoxide	83.8	6.2	0.07
Hexachlorobenzene	93.7	4.2	0.03
Mirex	97.9	13.6	0.20

Interlaboratory precision was test by total concentration of OCPs in human breast milk which was very low residual concentration in the milk sample compare to 13C labelled standards. Interlaboratory precision by HRMS of organochlorine pesticides was 35%, and accuracy was 100%, respectively. As compared with ongoing

precision and recovery of EPA1613 or 1668, 50-150% and recovery criteria, 25-150%, all analytical performance data were fully satisfactory at the analyte concentrations of interest.

Table 3 Results of interlaboratory study

	Total concentration (ng/g)										Average Concentration (ng/g)	Precision (%)	Total Precision (%)	Accuracy (%)	Total accuracy (%)
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10					
Lab. A	3.28	1.38	1.71	1.74	2.28	2.25	6.50	3.47	3.57	2.40	2.86	33.0	35.2	117.3	100.0
Lab. B	2.54	2.26	1.94	1.49	3.28	2.13	0.88	1.08	1.85	2.68	2.01	36.5		82.7	

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