ANALYSIS OF DIOXIN/FURANS IN BREAST MILK SAMPLES USING RAPID EXTRACTION AND HIGH RESOLUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMESTRY (HRGC/HRMS)

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Introduction

Dioxins (*Polychlorinated dibenzo-p-dioxins*, PCDDs) and furans (*polychlorinated dibenzofurans*, PCDFs) are among the most persistent organic pollutants (POPs) known - extremely tiny doses have been shown to cause negative health effects such as cancer, as well as to reproductive problems, abnormalities in fetal development, immune alterations, and disruption of hormones. Because dioxins and furans are attracted to fat and are resistant to metabolism, they are notorious for accumulating in the animals humans eat, and by that route accumulating in humans. Within the human body, the highest levels of these chemicals are in fat and breast milk. Due to that raw milk and its products are recognized to be a fine indicator of environmental exposure for persistent organic pollutants[1].

Determination of PCDDs and PCDFs in milk or other foodstuff is often a challenge task because of trace level in sample, and difficulty in sample preparation especially in extraction step. The constituent of milks generally is complex matrix consisting of fats, proteins, sugars, vitamins, and minerals, etc...[2,3] In order to efficiently separate the emulsified fat from this complex matrix, the extraction method requires scrupulously breaking the matrix structure of milk. Previously, the milk samples have often extracted by using conventional method such as liquid-liquid extraction or Soxhlet extraction. These methods are particularly time and labour consuming; moreover they require large amounts of organic solvents and laboratory space. Recently, some new extraction techniques such as accelerated solvent extraction (ASE), pressurized liquid extraction (PLE) have been developed and known with several advantages included reducing of solvent consumption, decreasing of extraction time and possibilities of automation[4].

In this study, an automatic PLE extraction technique was validated for efficient extraction of milk samples. This technique is relatively matrix independent and the main reason for an improved extraction speed is the possibility of using elevated temperatures and pressures. The system of manually dioxins and furans purification included of multi-silica gel column coupled with dual-layer active carbon column is applied for lipid removal, and finally the detection is carried out by using high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS). The method proposed in this paper is showed a capability of determination of trace level of dioxins and furans in animal milk and human milk also. Dioxins and furans in breast milk of primiparae who lived in Da Nang city, the South Central of Vietnam were preliminarily investigated based on new extraction, clean up and analysis techniques validated in our laboratory.

Materials and Methods

Validation study

The two commercial products of cow milk powder and one pool breast milk sample were selected as represented samples for validation test.

Pressurized Liquid Extraction: A PLE system (FMS, USA) with 3 sample modules of 100 mL extraction cell was used. The extraction pressure was set to 1700 psi, the practical temperature was 120°C, and the number of the static cycle was 2 for all experiments. Five (5) g of milk powder sample was mixed with anhydrous sodium sulphate and fill up to the extraction cell, the press of sample mixture is not necessary. Extractions were performed with n-hexane/dichloromethane/ethanol (5/2/1, v/v/v). Prior to all extractions the mixtures of ^{13}C labeled solutions comprised of 15 labeled dioxin/furan congeners (CIL, USA) were added in to the sample mixture to measure the efficiency of the extraction process[9]. The sample extract was evaporated for gravimetric

determination of the lipid content[5,6].

Lipid removal: The cleanup standard ^{13}Cl-2,3,7,8-TCDD (CIL, USA) is added to all extracts prior to cleanup to measure the efficiency of the cleanup process[6]. The sample extracts were defatted with concentrated sulfuric acid and further purified and fractionated on the multi-layer silica columns coupled with dual-layer activated carbon (Supelco)[7].

HRGC/HRMS analysis: The analyses of the seventeen dioxin/furans congeners were performed on a GC 7890A (Agilent) equipped with a High Resolution Mass Spectrometry (Autospec Premier, Waters) and a DB 5 MS capillary column (60 m x 0.25 mm I.D., film thickness 0.25 mm). The quantifications were based on the isotope dilution mass spectrometry, and the mass spectrometer was operated in the SIM mode, at the mass resolution of 10000.

Validation experiment: The studied method was comprehensively validated to confirm the capability for dioxins/furans quantification. The trueness was estimated via experiments of the fortified samples and cross-checking sample (with Eurofins laboratory, Hamburg, Germany).

Breast milk analysis

Breast milk samples (n=27) were collected from primiparae who were selected from the general population in four places in Da Nang city, Vietnam in 2011. Breast milks were collected in chemically cleaned amber glass containers and stored at -20^{o}C until analysis. Sample was dried by Unicryo MC6L freeze-drier (1 mbar, –50°C), (Uniequip, Germany) and homogenized by mortar and pestle. Homogenized sample (5 g) was used for treatment and analysis by validated method.

Results and Discussion

Validation results

Figure 1 shows the average recovery values of fifteen ^{13}C-PCDD/Fs surrogate standards and the ^{37}Cl-2,3,7,8-TCDD cleanup standard, they were 82 ± 15% and 85 ± 15%, respectively. The recovery of all standards are in the required range of 60~120%, complying with the EU regulation[8].

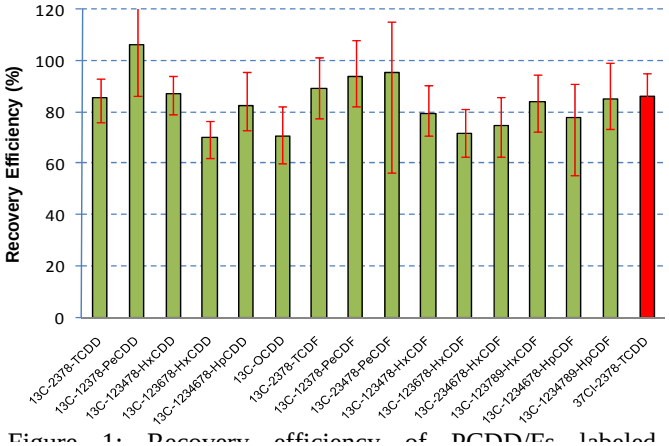


Figure 1: Recovery efficiency of PCDD/Fs labeled

Trueness of PCDD/PCDFs was estimated with fortified sample in natural cow milk matrix (n=7). Setting the fortified value as 100%, most analyzed congeners were between 95~117%, only one HxCDF and one HpCDF congener was up to 21% and 24% respectively (Figure 2). The repeatability was calculated for all analytes. RSDs were from 2~10% for all congeners (except 123789-HxCDD 18%).

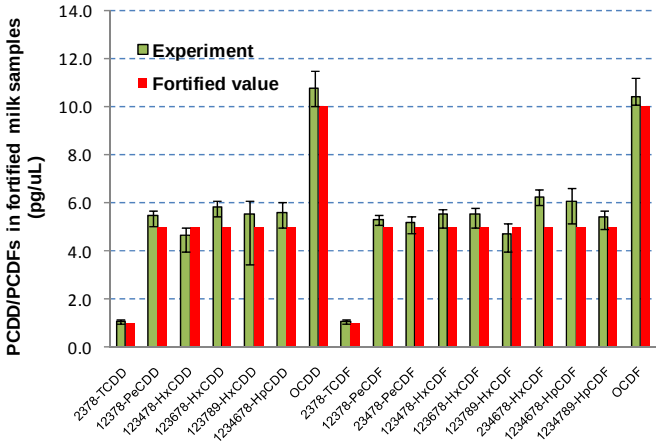


Figure 2: PCDD/PCDFs in fortified milk samples (n=7)

For the cross-checking sample, both extraction methods (PLE, Dioxin Lab., VEA vs. Liquid-Liquid, Eurofins GfA Lab., Hamburg) have found almost congeners' level belowed the quantification limit. However, the studied method gets better of lipid extraction recovery than the Liquid-Liquid extraction method used by Eurofins GfA Lab., Hamburg (Table 1). Method was proved the sensitivity with limit of detection (LOD) for analysis of milk samples, the value is 0.06 and 0.3 pg/g lipid of TCDD and PCDD/PCDFs-TEQ (WHO 2005), respectively. These values are far bellowed the EU-maximum level for dioxin/furans in milk and milk products (3.0 pg WHO-TEQ/g lipid) and similar those values mentioned in some previous reports[4,9].

Table 1: Fitness of cross-checking results

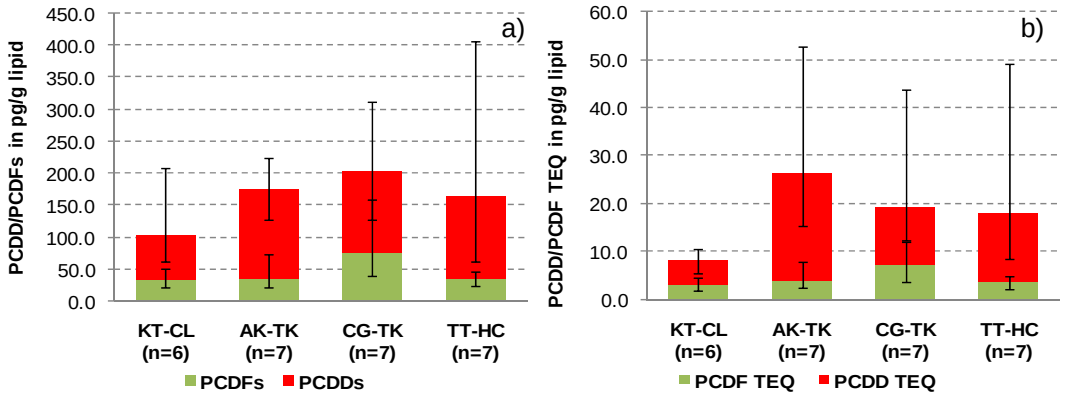
	Dioxin Lab., VEA	Eurofins GfA Lab.
Extraction method	PLE	L - L
Lipid recovery (%)	102	86
PCDD/F-TEQ (WHO 2005) in pg/g lipid:		
Lower bound, ND=0;	0	0
Upper bound; ND=LOD	0.3	0.2

(PLE: Pressurized Liquid Extraction; L - L: Liquid - Liquid Extraction; ND: Not detected)

Dioxins and furans in breast milks

Figure 3 illustrates the concentration of PCDDs and PCDFs in breast milk samples obtained from 27 primiparae coming from four wards located surround Da Nang airbase in Vietnam. Khue Trung ward, Cam Le district is located in the south of airbase, An Khe and Chinh Gian ward, Thanh Khe district in the west and north of airbase, and Hoa Thuan Tay, Hai Chau district in the east of airbase. The values were present in mass concentration in lipid weight base. The lowest PCDD/PCDFs concentration was found in Khue Trung ward, Cam Le district (102.8 pg/g) and the highest value is belonged to the Chinh Gian ward, Thanh Khe district (203.0 pg/g); other two places, An Khe and Hoa Thuan Tay ward have quite similar concentration of over 150 pg/g.

The rank order of TEQ concentration (WHO, 2005) was Khue Trung, Hoa Thuan Tay, Chinh Gian, An Khe and in range from 8.1~26.4 pg/g. In comparison with geographical position, the PCDD/PCDFs concentration and TEQs in population in An Khe, Thanh Khe district which is located nearest the contaminated sites in Da Nang hotspot airbase were significantly elevated compare with those in the location farthest from the contaminated sites, Cam Le district. These results appeared similarly with those results published in previous researches[10,11].



(KT, AK, CG, TT: Khue Trung, An Khe, Chinh Gian, Hoa Thuan Tay ward, respectively; CL, TK, HC: Cam Le, Thank Khe, Hai Chau district, respectively)

Figure 3: PCDD/Fs mass concentration[a)] and TEQ[b)] in breast milk

Figure 4 shows the PCDD/PCDFs congener profiles of four locations in this study. Congener profiles in three locations (An Khe[b)], Chinh Gian[c)], Hoa Thuan Tay[d)]) were found similar each other, and specified to those in hotspot areas as the recently publication of Tai T.P. et al[11].

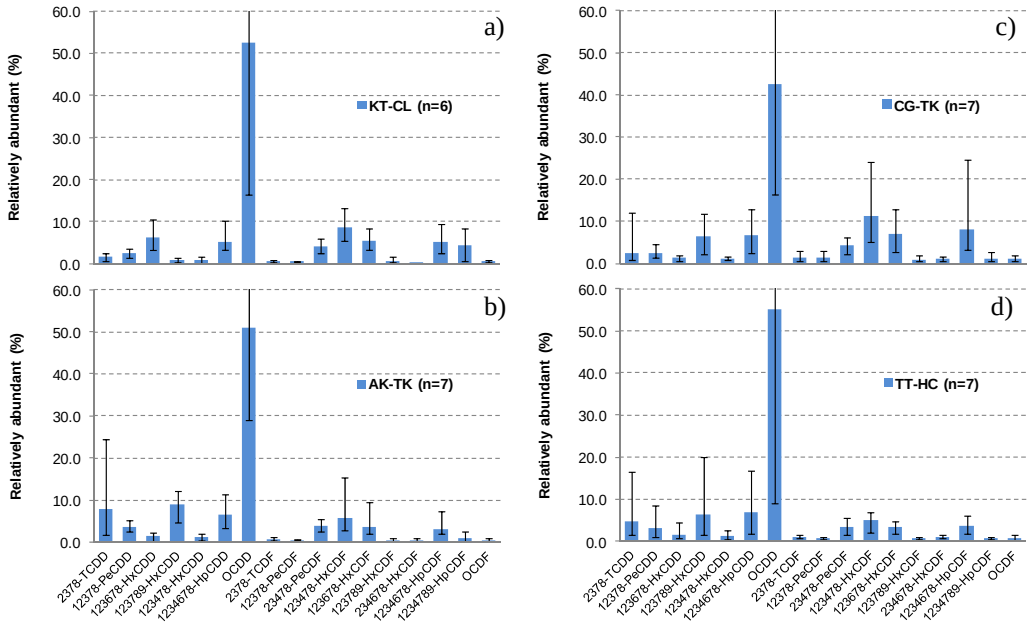


Figure 4: Relative abundant of dioxin/furans in breast milk

Acknowledgements
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References

1. EU. (2006); EC Regulation No. 1883/2006. Off. J. Europ. Union, L 364/32
2. Fürst P. et al. (1989); *Chemosphere*, 16: 1983-1988
3. Focant J.F. et al.(2005); *Organohalogen CompI*, 67
4. Goff D. (2010); *Dairy Science and Technology*
5. Supelco (2004); Instructions for Supelco Multi-Layer Silica Gel Column and Dual-Layer Carbon Reversible Colum.
6. Patterson et al. (1997); *CLIA Document, Environmental Health Laboratory, Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA:* 1–253
7. Schecter A. et al. (1991); *Chemosphere*, 23 (11-12): 1903-1912.
8. Tai T.P. et al. (2011); *Environ. Sci. Technology*
9. U.S. EPA. (1994); Method 1613B – Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.
10. U.S. EPA. (2000); Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds.
11. USDA (2011); National Nutrient Database for Standard Reference.