DEVELOPMENT OF ANALYTICAL METHOD FOR THE DETERMINATION OF PCDDs/Fs IN HUMAN SERUM BY HIGH RESOLUTION GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

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1. INTRODUCTION

Dioxin is commonly referred to 75 congeners of polychlorinated dibenzo-p-dioxins (PCDDs) and 135 congeners of polychlorinated dibenzofuzans (PCDFs) [6]. These compounds are highly toxic, persistent in environment, and are classified in the list of 26 substances belonging to groups of persistent organic pollutants (POPs) [2, 3]. Congener-specific analysis of PCDDs/Fs at trace and ultra-trace levels in samples often required highly sensitive, selective and precision methods such as high resolution gas chromatography (HRGC) in couple with triple quadrupole mass spectrometry and magnetic sector high resolution mass spectrometry (HRMS) [2, 4, 5].

While quantification of PCDDs/Fs in human blood samples has been well employed in developed countries, analytical procedures for this sample is limited in developing countries including Vietnam due to the lack of advanced analytical equipment and laboratory facilities. Human blood is among the most commonly used samples for evaluation of status of contamination and human exposure. Due to the low lipid content and small volume of serum or whole blood samples, quantification of PCDDs/Fs in these samples remains challenging in developing countries.

In this study, authors investigated analytical procedure for the determination of 17 toxic PCDD and PCDF congeners in human serum using HRGC-HRMS according to US EPA method 1613B with necessary modifications suitable for practical facilities in our laboratory in Vietnam-Russia Tropical Center (VRTC).

2. MATERIALS AND METHODS

2.1. Materials

Human serum samples, including blank samples which do not contain PCDDs/Fs obtained from National Institute of Standards and Technology (NIST), coded as HS1. Real samples (coded as HS2) were collected from individuals living in Southern Vietnam.

Chemicals: Solvents, chemicals purchased from Merck, Aldrich Sigma, which are at grade for gas chromatography.

Standards: All standards used for dioxins/furans analysis following US EPA 1613B: EDF-9999 (CS0.1, CS0.2, CS0.5, CS1, CS2, CS3, CS4 and CS5), EDF-8999, EDF-6999, EDF-5999, EDF-7999, EDF-4141, purchased from Cambridge Isotope Laboratories (CIL), USA. In order to improve the method quantification limit, an additional standard CS0.05 was prepared. This standard has similar constituent as CS1, in which concentrations of 17 native PCDD/F congeners were 20 times lower than those in the corresponding ones in CS1.

2.2. Analytical Methods

2.2.1. Investigations of method detection limit, accuracy and precision of the analytical procedure for the determination of 17 toxic congeners of dioxins/furans in human serum sample

The analytical procedure was conducted as follows:

- Prepare 5 HS1 serum samples (samples without PCDDs/Fs), 10 grams for each sample. Native standards from EDF-7999 containing 0.5 - 2.5 - 5.0 pg of 17 PCDD/F congeners and 0.5 ml ^{13}C-labelled PCDDs/Fs (EDF-8999) 2÷4 ng/ml were added to these 5 HS1 samples.

- 30 ml ethanol, 10 ml saturated $(NH_4)_2SO_4$ and 30 ml hexane were added in turn, and samples were extracted by shaker in 30 minutes. Hexane layer extract was descanted and extraction step was repeated. Two portions of hexane extract were combined and passed through anhydrous Na_2SO_4 to remove water. The extract was then subjected to an activated charcoal column.

- Determination of lipid content: An aliquot of hexane extract (10% of total volume) was added to petri dish and lipid content was determined by gravimetric method.

- Fractionation of PCDDs/Fs in activated carbon column AX-21. The extract was subjected to activated carbon column AX-21 containing 200 mg mixture of AX-21 and Celite (1:9). PCDDs/Fs were eluted with 7 ml heated toluene at 120^{o}C. The eluate was concentrated to 50 µl and 50 ml hexane was added for the subsequent clean-up through multilayer column.

- Clean-up by multilayer column. The activated carbon column eluate was added to multilayer column containing 3 layers of H_2SO_4-impregnated SiO_2 and 1 layer of KOH-impregnated SiO_2. PCDDs/Fs were eluted with 50 ml hexane. The eluate was then concentrated to about 20 ml.

- Clean-up by alumina column. Sample extract was then passed through 4 grams alumina column. Alumina was activated at 600^{o}C for 24 hours. Interference compounds were separated with hexane and mixture of hexane : dichloromethane (95:5). PCDD/F fraction was eluted with mixture of hexane : dichloromethane (1:1).

- Sample extract after alumina column was spiked with ^{13}C-labelled standards (EDF-5999) containing 1÷2 ng PCDDs/Fs. Extract was then concentrated to 20 µl and injected into HRGC-HRMS for quantification.

2.2.2. Analysis of real samples

Total 5 batches of samples were analyzed in 2015. Each batch contains 18 samples including 16 real samples, 1 blank, and 1 spiked matrix sample (SPM sample). SPM sample was similarly treated as those described in section 2.2.1, except the amount of native standard EDF-7999 to be added to HS1 sample was 20-100-200 pg/sample, respectively. Instrumental analysis of PCDD/F congeners according to those described in section 2.2.4.

2.2.3. Cross checking analysis

Five serum samples coding A, B, C, D and E were sent to Eurofins Laboratory in Germany for cross checking in 2015.

2.2.4. Instrumental quantification of 17 PCDD/F congeners by HRGC-HRMS

Concentrations of PCDD/F congeners were analyzed by high resolution gas chromatography (Agilent GC 7890A) couple with high resolution mass spectrometry (Autospect Premier, Waters, USA) with DB-5MS capillary column (60 m x 0.32 mm x 0.25 μm). Mass selective detector has resolution of ≥ 10000 and monitored using SIM mode for m/z of PCDD/F congeners.

3. RESULTS AND DISCUSSION

3.1. Calibration curve

Calibration curve was constructed based on 9 points from standard solutions from CS0.05 to CS5 (described in section 2.1). The correlation coefficient was $r^2 >$ 0.999 and RSD < 13.0%, which meet the criteria values ($r^2 > 0.99$ and RSD < 20%) [4]. The linearity of calibration standards as follow: TCDD/TCDF: 0.025÷200 ng/mL, Penta- to Hepta-CDD/CDF: 0.125÷1000 ng/mL, and OCDD/OCDF: 0.25÷2.000 ng/mL.

3.2. Limit of detection and limit of quantification

Result of method validation experiments using 5 repetitive samples spiked with 0.5 - 2.5 - 5.0 pg/sample of 17 PCDD/F congeners (section 2.2.1) is given in Table 1.

The accuracy of the method was evaluated based on the recovery of native standards (EDF-7999) added to the sample. Table 1 showed that the recovery values ranged from 88.6 to 118%. The precision was evaluated based on the relative standard deviation (RSD) with the criteria ≤ 20% recommended by US EPA [4, 5]. Result in table 1 showed that the RSD ranged from 2.9 to 9.51%, which meet the criteria.

The limit of quantification (LOQ) of the method corresponded to the lowest point of calibration curve (CS0.05). This is because at these concentrations all the parameters met the criteria: the signal/noise > 5, the recovery of all analytes ranged from 88.6÷118%, and RSD < 20% [1, 4, 5]. The LOQ of the congeners are as follows: Tetra-CDD/CDF: 0.5 pg/sample, Penta- to Hepta-CDD/CDF: 2.5 pg/sample and OCDD/OCDF: 5.0 pg/sample.

The limit of detection (LOD) values were calculated according to the formula: LOD = 3.747 x SD, in which SD is standard deviation of LOQ investigation experiment (table 1).

Table 1. Result of method validation experiments (n=5, t = 3,747)

Analyte	Fortified (pg/g)	Average found value (pg/g)	Recovery (%)	Standard Deviation (pg/g)	Relative standard deviation (%)	LOD (pg/g)
2,3,7,8-TCDD	0.05	0.057	115	0.002	3.40	0.007
1,2,3,7,8-PeCDD	0.25	0.247	98.9	0.011	4.44	0.041
1,2,3,4,7,8-HxCDD	0.25	0.233	93.4	0.011	4.82	0.042
1,2,3,6,7,8-HxCDD	0.25	0.265	106	0.018	6.74	0.067
1,2,3,7,8,9-HxCDD	0.25	0.246	98.5	0.014	5.70	0.053
1,2,3,4,6,7,8-HpCDD	0.25	0.271	109	0.010	3.87	0.039
OCDD	0.50	0.592	118	0.052	8.81	0.195
2,3,7,8-TCDF	0.05	0.049	97.6	0.003	6.70	0.012
1,2,3,7,8-PeCDF	0.25	0.250	99.9	0.020	8.00	0.075
2,3,4,7,8-PeCDF	0.25	0.237	94.6	0.013	5.33	0.047
1,2,3,4,7,8-HxCDF	0.25	0.254	102	0.010	3.78	0.036
1,2,3,6,7,8-HxCDF	0.25	0.236	94.3	0.007	2.91	0.026
1,2,3,7,8,9-HxCDD	0.25	0.237	94.9	0.016	6.70	0.060
2,3,4,6,7,8-HxCDF	0.25	0.241	96.3	0.023	9.51	0.086
1,2,3,4,6,7,8-HpCDF	0.25	0.225	90.0	0.011	4.80	0.040
1,2,3,4,7,8,9-HpCDF	0.25	0.222	88.6	0.008	3.49	0.029
OCDF	0.50	0.546	109	0.044	8.05	0.165

3.3. Analysis of real samples and cross-checking samples

Human serum samples were analyzed following the procedure which were validated as described earlier. Each experiment batch comprises 16 real serum samples, 1 blank and 1 SPM sample. Native standard for 17 PCDD/F congeners from EDF-7999 was spiked to SPM at the amount of 20-100-200 pg/sample.

Result in figure 1 showed that the average recovery of native standards for 5 SPM samples ranged from 93.5 to 112.3%. Fifteen ^{13}C-labelled PCDD/F standards from EDF-8999 were spiked at levels of 1÷2 ng/sample. Their average recoveries for 80 serum samples ranged from 61.1 to 104.1% (figure 2).

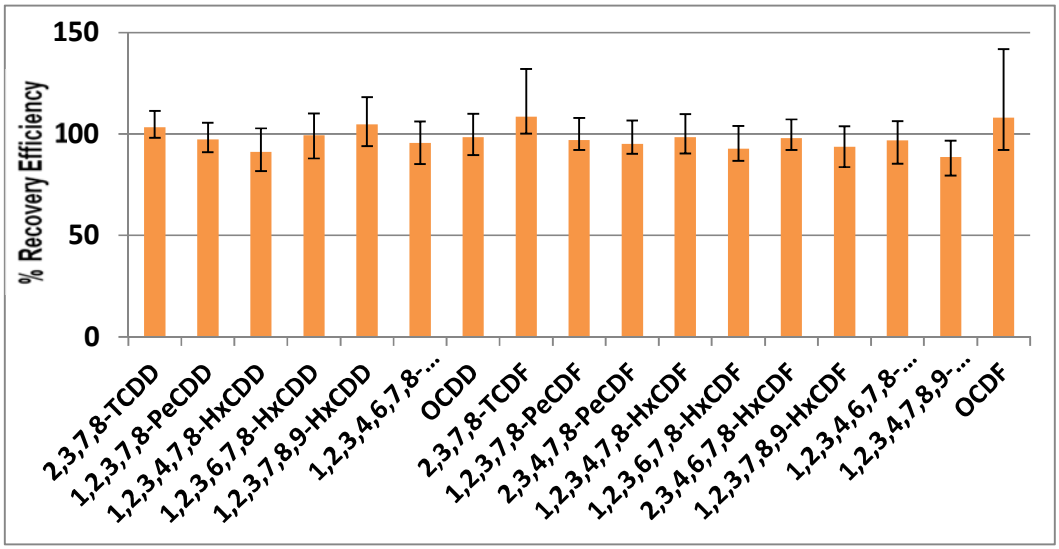

Figure 1. Recovery of native standards in SPM samples

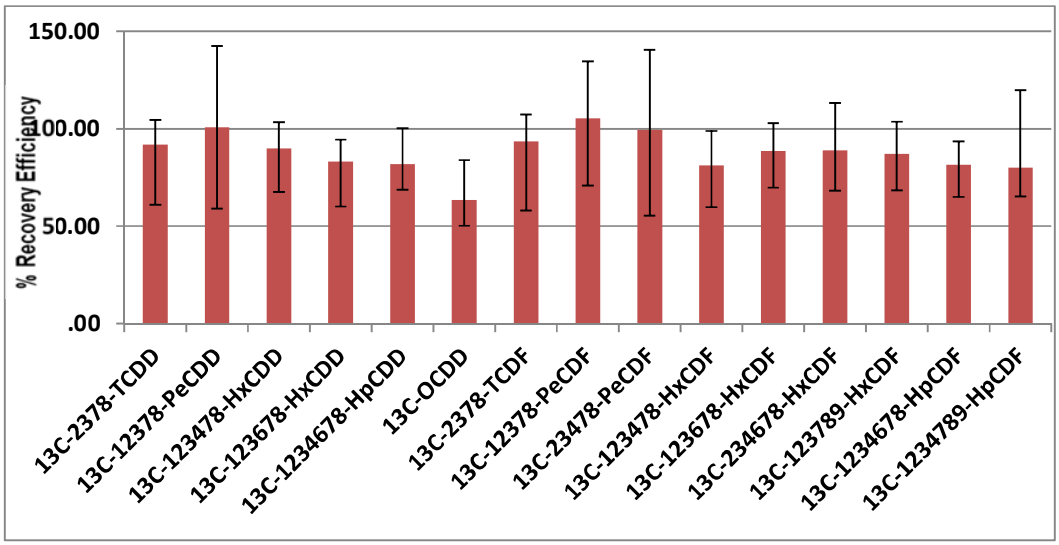

Figure 2. Recovery of labelled standards in real samples

Cross-checking analysis: Five serum samples were randomly selected and sent to Eurofins Laboratory in Germany for comparison among two laboratories. Result of cross-checking analysis is given in table 2.

Table 2. Result of cross-checking analysis between VRTC and Eurofins laboratories

Sample code	VRTC (Vietnam)		Eurofins (Germany)		RPD (%)	
	Lipid (%)	WHO-TEQ 2005 Upper bound (pg/g lipid)	Lipid (%)	WHO-TEQ 2005 Upper bound (pg/g lipid)	Lipid (%)	WHO-TEQ 2005 Upper bound (pg/g lipid)
A	0.857	57.8	0.918	58.8	6.87	1.72
B	1.12	90.8	1.06	74.1	5.5	20.3
C	0.621	34.6	0.682	32.1	9.36	7.5
D	0.469	85.4	0.564	66.3	18.4	25.2
E	0.905	102	0.842	81.0	7.21	22.9

Due to very low levels of PCDDs/Fs, the difference between two laboratories could be ≤ 40%. Result in table 2 indicates good agreement of the two laboratories, proving the full capability of ultra-trace levels analysis of PCDDs/Fs in human serum in laboratory at VRTC.

4. CONCLUSION

In summary, authors successfully investigated and validated an analytical procedure for determination of 17 toxic PCDD/F congeners based on US EPA method 1613B with necessary modifications to suite practical facilities in laboratory in VRTC, Hanoi, Vietnam. Authors results (the accuracy; precision; detection and quantification limit and the cross-checking analysis) clearly demonstrate the suitability of the method for the determination of PCDDs/Fs at ultra-trace levels in human serum samples.

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TÓM TẮT

PHÁT TRIỂN PHƯƠNG PHÁP PHÂN TÍCH XÁC ĐỊNH HÀM LƯỢNG PCDD/PCDF TRONG HUYẾT THANH NGƯỜI BẰNG THIẾT BỊ SẮC KÝ KHÍ PHÂN GIẢI CAO - KHỐI PHỔ PHÂN GIẢI CAO

Bài báo trình bày các bước khảo sát, xây dựng phương pháp phân tích 17 đồng loại độc PCDD/PCDF trong huyết thanh người bằng thiết bị sắc ký khí - khối phổ phân giải cao (HRGC/HRMS) trên cơ sở cải biến phương pháp US EPA 1613B của Cục Bảo vệ Môi trường Hoa Kỳ cho phù hợp với điều kiện Phòng thí nghiệm Trung tâm Nhiệt đới Việt - Nga. Kết quả khảo sát cho thấy tất cả các thông số của phương pháp (giới hạn phát hiện, giới hạn định lượng, độ đúng, độ lặp) đạt được đều đáp ứng đầy đủ các yêu cầu của phương pháp EPA 1613B. Phương pháp phân tích này đã được sử dụng để phân tích các mẫu thật và phân tích kiểm tra chéo 5 mẫu với với Phòng thí nghiệm Eurofins của Đức. Kết quả kiểm tra chéo cho thấy sự khác biệt của các kết quả phân tích giữa 2 phòng thí nghiệm trong khoảng 1,72÷25,2% và hoàn toàn nằm trong khoảng cho phép (dưới 40%) về độ lệch giữa các phòng thí nghiệm.

Từ khóa: Phân tích PCDD/PCDF, huyết thanh người; Analysis of PCDDs/PCDFs, human serum.

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