Development and validation of GC×GC–MS/MS method for the determination of PCDD/Fs and dl-PCBs in human serum

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Introduction

Human serum from peripheral environment is an ideal matrix to monitor the internal exposure of various chemicals. Due to the very low lipid content, there were still challenges for the analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in human serum. The GC×GC system could provide higher chromatographic peak capacity and higher separation efficiency compared to one dimensional GC system [1,2], and offer better performance in simultaneous determination of complex chemical mixtures. Furthermore, up to date triple quadrupole analyzers (MS/MS) achieved a high-speed scanning, which made it a suitable detector for GC×GC. MS/MS, which could offer enough sensitivity and selectivity, is a good alternative to HRMS for detecting dioxin and dioxin like compounds [3, 4]. In the present study, a method using GC×GC-MS/MS was developed to simultaneously determine 17 2,3,7,8-subsitituted PCDD/F congeners and 12 dl-PCB congeners in human serum by one single injection.

Materials and methods

Chemicals and Instruments

All of standard solutions were purchased from Wellington Laboratories (Guelph, Canada). All solvents were ultra resi-analyzed grade (J.T.Baker, Cen-ter Valley, PA, USA). Diatomite was purchased from Merck KgaA (Darmstadt, Germany).

A GC×GC-MS/MS (Shimadzu GCMS-TQ8040, Japan) with a Zoex KT2004 loop type modulator (Zoex Corporation, USA) was employed for this study. Electronic refrigeration equipment was used for the cold jet and nitrogen was used as purge gas for the hot jet. GC-MS post-run analysis software (GC Image 2.2.2, Shimadzu, Japan) was used for the raw data analysis. GC Image software (Zoex Corporation, USA) was used for the data analysis in contour plots (2D chromatogram). Two GC columns in the system were combined by a modulator-demodulator. DB-5MS (30 m, 0.25 mm ID×0.25 μm, Agilent Corporation, USA) and BPX50 (2.5m, 0.1mm ID×0.1μm, SGE Corporation, Auatralia) were used as the primary and secondary columns, respectively, which were connected by a glass connector. 2 μL of the sample extract was injected into the split/splitless inlet.

The results of real human serum obtained from GC×GC-MS/MS were compared with the results from a Trace 1300 Gas Chromatography (Thermo Sci-entific, Milan, Italy) equipped with a DB-5MS capillary column (60 m × 0.25 mm i.d.× 0.25_m) and coupled to a HRMS (DFS, Thermo Scientific, Bremen, Germany). The instrument parameters were described elsewhere [5]

Sample preparation

Sample extraction and clean-up were performed according to our previous study [5], with some modification. Briefly, approximately 5 g of serum sample was freeze-dried before being blended with free crystalline silicic acid (EXtrelutNT, Merck KGaA, Germany). After spiking with $^{13}C_{12}$-labeled internal standards, samples were extracted using Accelerated Solvent Extractor (ASE300, Dionex, USA). The bulk lipid was removed by shaking with acid-modified silica-gel, and further cleanup was achieved using a Power Prep instrument (Fluid Management Systems, Waltham, MA, USA). Then, the fractions containing PCDD/Fs and PCBs were collected and concentrated by nitrogen blowing to approximately 20 μL. Before the instrumental analysis, the $^{13}C_{12}$-labeled injection standards were added to the final extract.

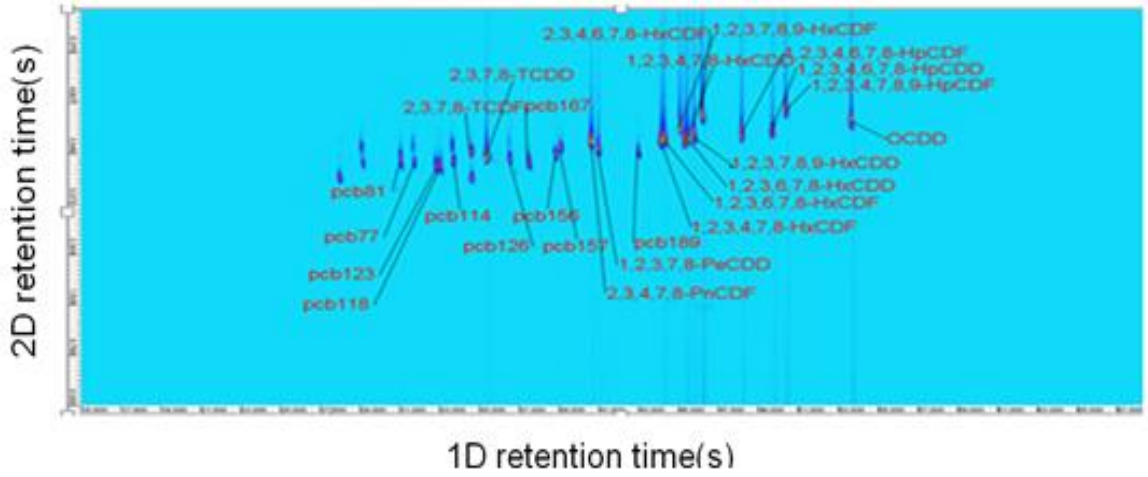

Figure1 the separation of all 29 targets compounds by GC×GC-MS/MS

Results and discussion

Instrument Conditions

The modulator is the key element of a GC×GC system, which accumulates and focuses fractions eluting from the first column and re-injects them into the second column. For modulator, cold jet flow rate is a key parameter. The suitable cold jet flow rate could improve the detection results of instrument. After optimization, the cold jet flow rate of 7.5 L/min was chosen for all subsequent experiments. Modulation period is another critical factor for the separation of the target compounds. It must be set within an appropriate range in order to meet the separation requirements of the two-dimensional columns. When dealing with the columns set, difficulties were found in assigning peak areas for some HxCDD compounds, as there was a considerable overlap between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. To solve this problem in the second dimension of separation, 6s of modulation period was chosen.

Table 1. Quantitative results obtained for the PCDD/Fs and dl- PCBs in real samples (pg/g)

	Sample1		Sample2	
	HRGC-HRMS	GC×GC-MS/MS	HRGC-HRMS	GC×GC-MS/MS
2378-TCDD	n.d.	n.d.	n.d.	n.d.
2378-TCDF	n.d.	0.12	n.d.	0.09
12378-PeCDD	0.42	0.33	0.57	0.36
12378-PeCDF	20.16	18.41	18.92	16.6
23478-PeCDF	n.d.	n.d.	n.d.	n.d.
123478-HxCDD	0.21	0.17	0.17	0.12
123678-HxCDD	0.21	0.15	0.17	0.11
123789-HxCDD	0.22	0.17	0.26	0.11
123478-HxCDF	0.09	0.07	0.12	0.12
123678-HxCDF	0.09	0.07	0.12	0.12
234678-HxCDF	1.63	0.07	0.06	0.09
123789-HxCDF	n.d.	n.d.	0.57	0.41
1234678-HpCDD	16.79	14.05	12.65	2.87
1234678-HpCDF	0.05	0.2	0.32	0.16
1234789-HpCDF	n.d.	n.d.	1.43	0.36
OCDD	415.92	388.39	56.77	77.21
OCDF	n.d.	n.d.	n.d.	0.55
PCB77	2.3	1	3.56	1.46
PCB81	n.d.	n.d.	0.42	0.73
PCB105	7.63	5.9	33.98	22.64
PCB114	2.82	1.82	7.65	5.24
PCB118	32.16	22.44	79.53	59.74
PCB123	2.72	1.43	2.49	1.39
PCB126	n.d.	0.16	1.26	0.51
PCB156	17.56	10.89	24.58	15.07
PCB157	1.92	1.71	8.63	2.58
PCB167	4.75	3.95	6.92	5.45
PCB169	n.d.	0.25	n.d.	0.2
PCB189	1.62	0.94	1.48	1.05
Total TEQs	1.57	1.25	1.58	1.09

Multiple reactions monitoring (MRM) mode was used in MS/MS. Based on the specific criteria for

confirmatory methods of dioxin and dioxin like PCBs in food and feeds released by The European Commission [6], two MRM transitions were monitored for each target for quantitation and qualification. Quantitation was performed with the quantitation transition only and the qualification transition was exclusively used to verify ion ratio for target identification.

After optimization of GC×GC-MS/MS, the separation of all 29 targets compounds in serum sample was shown in Fig.1.

Method validation

Quantification is based on isotope dilution according to EU regulation [6]. The linearity of GC×GC-MS/MS method was evaluated in the ranges of 0.1~100 ng/mL and 0.1~200 ng/mL from duplicate measurements of a five-point calibration curve (EPA1613CVS CSL-CS3 for PCDD/Fs and P48-W-CVS CS1-CS5 for dl-PCBs). The correlation coefficients of the calibration curves were in a range of 0.9949-0.9999, showing a good linearity throughout the concentrations range. The LODs of this method were individually defined as the concentration needed to produce a signal-noise ratio of 3:1 by analysis of 5 mL spiked serum. The LODs of PCDD/Fs ranged from0.02 to 0.8pg/g, and the LODs of dl-PCBs were in the range of 0.1-0.4 pg/g.

To test the accuracy and reliability of GC×GC-MS/MS, two human serum samples were detected by both GC×GC-MS/MS and GC-HRMS for target PCDD/Fs and dl-PCBs. The concentration values for the most of targets and total TEQs values for these two samples from these two methods were comparable as shown in table 1.

Acknowledgements

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