Validation of a GC-MS method for indictor PCBs in marine fish and its application on survey in Beijing

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Introduction

The official announcement made by Belgian authorities in May 1999 that samples of animal feed contained high concentrations of dioxins once again raised consumer concern on food safety issues. It was discovered that the samples contained increased amounts of polychlorinated biphenyls (PCBs), and that they were contaminated by commercial mixtures of PCBs (i.e. Aroclor 1254 and Aroclor 1260). Subsequent analysis of various food samples, for example deriving from pork and chicken, revealed severe contamination by PCBs, sometimes exceeding the tolerance level set by the European Commission for the sum of seven indicator PCBs (28, 52, 101, 118, 138, 153 and 180) that is based on abundance, chromatographic resolution, response and availability as a standard, but not on toxicological considerations. This called for immediate action to solve the crisis with increasing demands on analytical laboratories to perform fast and reliable PCB analysis of numerous food and animal feed samples. In the last few years, more ^{13}C-labelled reference standards have become commercially available. As a result, the isotope dilution technique is also becoming the method of choice for the analysis of PCBs. Less information is available on background level of indictor PCBs in food in China, because currently method with GC-ECD is most commonly used in analytical practice in China. The aim of this paper is to validate an isotope dilution GC-MS method for determination of indicator PCBs in marine fishes and determinate 7 indicator PCBs in some marine fishes from supermarkets in Beijing, China.

Method and Materials

Reagents and samples

PCBs standards solutions including labeled internal standard (13C-PCB28, 52, 118, 153, 180, 202, 206, 209), recovery standard (13C-PCB101, 194), PAR standard (PCB 18, 28, 33, 52, 44, 70, 101, 118, 105, 153, 138, 128, 187, 180, 170, 199, 195, 194, 206, 209) and calibration standard (20-2000ng/ml) were purchased from Wellington Laboratories. Window defining standard were purchased from Cambridge Isotope Laboratories Co.

Peanut oil was selected as matrix substitute for LOD value. One fish sample free from PCBs contamination was selected for validation experimental.

Fish sample were collected from 4 supermarkets in Beijing city.6 samples were full fish and 3 samples were processed fish meat. The species of fish involved were commonly eaten by local people and included Trichiurushaumela (3 samples), Pseudosciaena polyactis (2 samples),

Pseudosciaena crosea (1 samples), Pampus argenteus (1 samples), Pagrosomus major (1 samples), Miichthys miiuy (1 samples).

Instrument and method

All the samples were analyzed by HRGC-LRMS (MD800, Fison Co) and CP8 capillary column (30m×0.32mm×0.25μm).The GC temperature program was 100 (held for 1min) increased at 15 /minto180 increase at 3 /min to 240 then increase at 10 /min to 285 (held for 10min) Injector temperature was 250 and injection was made on splitless mode (1 min).Helium at a pressure of 5 psi was used as carrier gas. MS was operated in EI (70eV),source and transferline temperature were set at 250 and 290 , respectively. Monitored masses in SIM mode were showed in Table 1. Quantification was made by stable isotope dilution.

Table 1 monitored masses in SIM mode

Congeners	Mass	Type of mass	Ratio of mass	Congeners	Mass	Type of mass	Ratio of mass
T_3CB	256/258	M/M+2	1.03±20%	13C-T_3CB	270	M+2	1.03±20%
T_4CB	290/292	M/M+2	0.78±20%	13C-T_4CB	304	M+2	0.78±20%
P_5CB	324/326	M/M+2	0.62±20%	13C-P_5CB	338	M+2	0.62±20%
H_6CB	358/360	M/M+2	0.52±20%	13C-H_6CB	372	M+2	0.52±20%
H_7CB	394/396	M+2/M+4	1.04±20%	13C-H_7CB	406	M+2	1.04±20%
O_8CB	428/430	M+2/M+4	0.89±20%	13C-O_8CB	442	M+4	0.89±20%
N_9CB	462/464	M+2/M+4	0.78±20%	13C-N_9CB	476	M+4	0.78±20%
$D_{10}CB$	498/500	M+4/M+6	1.17±20%	13C-$D_{10}CB$	510	M+4	1.17±20%

Extraction

Soxhlet extraction was used for the sample extract. Mixture of samples and sodium sulfate were added ^{13}C labeled internal standard and were extracted for 18 hours at least with hexane/dichloromethane (1:1).

Clean up

After evaporation, extract was cleaned on a multi-layer silica column (4g anhydrous sodium sulfate, 4g activated silica gel, 10g 44%H_2SO_4 silica gel, 2g activated silica gel, 4g anhydrous sodium sulfate). PCB were eluted with 180ml hexane. Extract was concentrated up to 1ml and the PCB were separated on a alumina column (2.5g basic alumina oxidate). The column was eluted with 30ml hexane(15ml*2) and 20ml 95% DMC/hexane. Combined elution was concentrate to about 50ul and added recovery standard, then analyzed by GC-MS.

Validation

Validation experimental was made by spiked fish sample analysis. The blank fish sample of 6 was spiked with PAR standard (spiking level 1ng/g), then was analyzed. Accuracy and precision were expressed with recovery and RSD respectively. LOD is defined as minimum concentration of PCB in the sample that will produce clearly defined peaks with a S/N ratio equal to three. LOD is obtained by analyzing matrix substitute peanut oil and calculated as follow:

$$MDL_r = \frac{3*N*M_S}{H*RRFn*S}$$

N-noise
M_S –amount of labeled internal standard,
H-peak high of the labeled internal standard.
S-amount of sample ,
RRF_n-reletive response factor.

Determination of fish samples

All the edible parts of fishes were removed and ground then frozen dry by frozen dryer. About 4g dry powder of samples were mixed with 10g anhydrous sodium sulfate then were analyzing.

Results and Discussion

Results of validation were shown in table 2-3. Recoveries for internal standards ^{13}C-PCB were used, $^{13}C_{12}$-PCB28 within 53.9-80.8%, $^{13}C_{12}$-PCB52 within 53.9-81.2%, $^{13}C_{12}$-PCB118 within 64.1-90.4%, $^{13}C_{12}$-PCB153 within 56.6-81.1%, $^{13}C_{12}$-PCB180 within 55.5-77.3%, $^{13}C_{12}$-PCB202 within 54.3-75.6%, $^{13}C_{12}$-PCB206 within 54.0-76.4%, and $^{13}C_{12}$-PCB209 within 50.0-72.9%. Results showed that the method was suitable for determination of indicator PCB in marine sample.

Table 2 Result of recovery and RSD

Congeners	Recovery %	RSD% n=6	Congeners	Recovery %	RSD% n=6
PCB18	79.7	3	PCB138	99.7	3
PCB28	95.9	7	PCB128	101.8	4
PCB33	92.2	8	PCB187	91.6	3
PCB52	91.8	6	PCB180	92.2	3
PCB44	106.0	6	PCB170	101.0	2
PCB70	112.6	5	PCB199	99.3	4
PCB101	84.3	3	PCB195	105.7	4
PCB118	89.7	1	PCB194	102.1	3
PCB105	106.6	5	PCB206	95.0	2
PCB153	94.8	2	PCB209	97.4	2

Table 3 LOD of PCB

Congeners	LOD ng/g	Congeners	LOD ng/g	Congeners	LOD ng/g
PCB18	0.009	PCB118	0.004	PCB170	0.011
PCB28	0.008	PCB105	0.012	PCB199	0.008
PCB33	0.005	PCB153	0.022	PCB195	0.008
PCB52	0.012	PCB138	0.017	PCB194	0.012
PCB44	0.008	PCB128	0.006	PCB206	0.005
PCB70	0.004	PCB187	0.007	PCB209	0.005
PCB101	0.014	PCB180	0.014		

Determination of fish samples

PCBs can be determinated in all the samples. The total concentration in wet weight basis for 9 fish samples was found from 0.19 to 4.42 ng/g wet weight and the concentration of 7 samples were lower than 1 ng/g. Figure 1 show that results. Results showed that PCBs in samples were far lower than 2 mg/kg the maximal level set up by China recently.

. Figure 1. Total concentration of indicator PCBs in samples

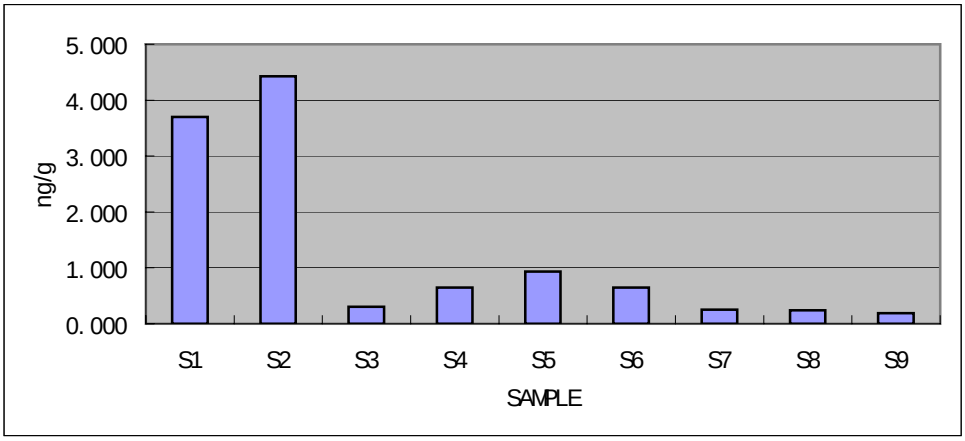
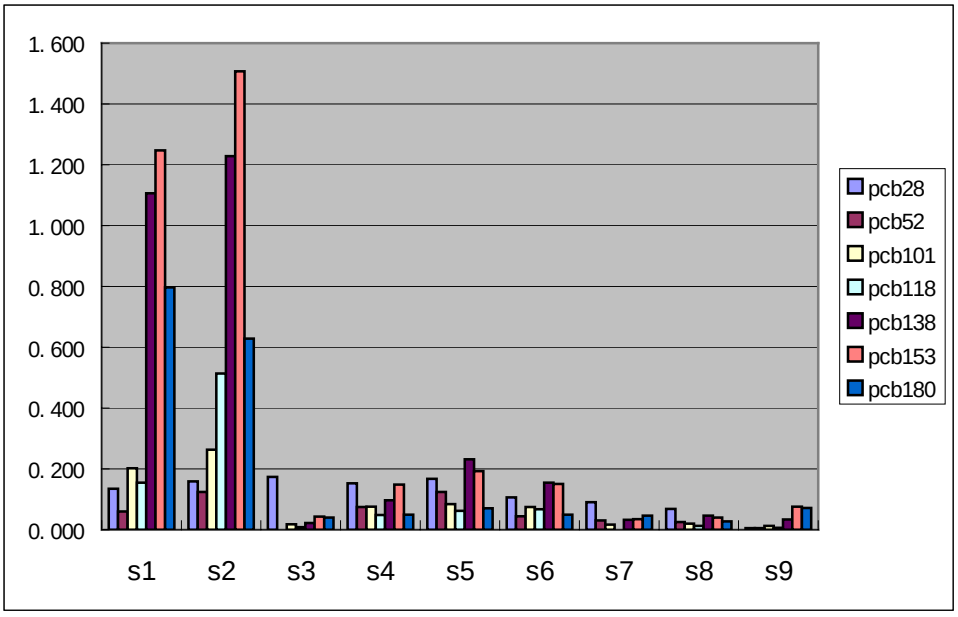


Figure 2. Profile of 7 indicator PCBs in samples (ng/g)



The indicator PCB profile was also analyzed in the 9 samples. Fig2 showed the profiles of PCBs in 9 samples. From sample to samples the pattern of PCB is different. The concentration of PCB28 was the highest in 4 samples, PCB138 in 2 samples, PCB153 in 3 samples . The

concentration of PCB52 and PCB118 were the lowest in 5 samples and 4 samples respectively. In all of samples PCB138 and PCB 153 were main contributors to the total concentration of PCBs. The Sum of PCB138 and PCB153 were 21.3% to 63.6% of total concentration in 9 samples.

The total concentration of sample 1 and sample 2 were much higher than other samples and their pattern were similar. These two samples were fished from the same sea area and were processed by the same manufacture.

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Reference

[1] Reference Method for the Analysis of Poly chlorinated Biphenyls (PCBs), Report EPS 1/RM/31 March, 1997.