

THE RESULT OF THE LARGE SCALE DETERMINATION OF PCDDs, PCDFs AND COPLANAR PCBs IN POLISH FOOD PRODUCT SAMPLES USING GC-MS/MS TECHNIQUE

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Introduction

Since June 1999 food products in Poland were analysed in our Laboratory for PCDDs, PCDFs and coplanar PCBs as a result of so called "Belgian chicken dioxin contamination problem". Food samples were extracted to obtain fat, which was cleaned-up using two methods: the carbon column method and semipermeable membranes for fat removal. The use of semipermeable membranes (SPM) for fat removal in trace analysis is recently widely used method for fast removing fat from the sample¹. The subject of analysis were about 400 samples of: milk, powdered milk, dairy products, eggs, meat, vegetable oils, fishes and fish oils as well as animal feeding material components as animal and fishmeal.

Samples of fresh food products were sampled and delivered to our laboratory from the representative territorial Veterinary Inspectorates.

Experimental

Solvents like hexane, dichloromethane, toluene, ethyl acetate, methanol, cyclohexane and acetone of „Ultra Resi[®]” purity, anhydrous sodium sulphate and sulphuric acid were purchased from J.T.Baker (Deventer, The Netherlands).

Active carbon for chromatography 0.3+0.5 mm, Alumina basic, activity I and Alumina acidic activity I, silicagel 60 were purchased from Merck (Darmstadt, Germany) Active carbon was purified by extraction with toluene in Soxhlet apparatus for at least 7 days and then dried in vacuum at 200°C

SPM membranes of 25 mm x 300 mm of 80 µm polyethylene wall thickness were of EST, St. Joseph, MO, California USA, purchased from LABICOM, Czech Republic.

Water of HPLC purity was used without further purification.

Native and isotopically labelled ¹³C-PCDDs/Fs/PCBs and ³⁷Cl-TCDD standard solutions were purchased from Wellington Laboratories, Guelph, Ontario, Canada.

Spiking solution in acetone was prepared by dilution and mixing of concentrated ¹³C-PCDDs/Fs EPA 1613 LCS and coplanar ¹³C-PCB MBP-CP standards obtaining final concentration of 1 ng/ml of the individual congener (except of 2ng/ml of OCDD)

GC-MS/MS system

ThermoQuest GCQplus GC-MS/MS systems were used for analysis. Mass selective detectors were adjusted in double fragmentation MS/MS mode. The secondary ion signals were recorded separately for PCDDs, PCDFs and coplanar PCBs.

Two different columns were used for chromatographic separation of individual congeners: DB-5MS (60m, 0,25 mm) as routinely used, low polarity capillary column for separation of PCDDs and PCBs and DB-17 (30m, 0,25 mm) intermediate polarity column which performance is suitable for separation of PCDFs, mainly 2,3,7,8-TCDF and P₃CDFs.

Sample purification – Method I (SPM method):

Samples after weighing (5g of fat content), spiking with 1 ml of ^{13}C -PCDDs/Fs/PCBs acetone solution were freeze dried or mixed with sodium sulphate and extracted in Soxhlet apparatus with dichloromethane for 16 hours. After solvent evaporation fat content was recorded. Then, the fat was dissolved in 40% dichloromethane in hexane (SPM solvent), placed in SPM membrane and dialysed with 80 ml portion of the same solvent for 24 h. Animal and vegetable fat samples were spiked, dissolved in SPM solvent, placed in SPM membranes and dialysed for 24h. The sample was concentrated in rotary evaporator and cleaned up in a multilayer silica column (from bottom to top: glass wool, silica 0,5g, sodium hydroxide-silica 5g, silica 0,5g, sulphuric acid-silica 8g and sodium sulphate 2g) eluted with 100 ml of hexane. Afterwards, the extract was purified in a combined basic/acidic Alumina column filled up with 2g of each of the sorbent freshly activated sorbent overnight in 600°C . The column was eluted with 60 ml of 2% dichloromethane in hexane containing coplanar mono-ortho PCBs, and 50 ml of 50% dichloromethane in hexane containing PCDDs, PCDFs and coplanar non-ortho PCBs. The collected fractions were concentrated up-to 1 ml and 20 μl of tetradecane $^{13}\text{C}_{12}$ -1234-TCDD and $^{13}\text{C}_{12}$ -123789-HxCDD solution was introduced. Afterwards the sample volume was reduced to 20 μl in a stream of nitrogen.

Sample purification – Method II (carbon column clean-up):

Extract obtained from Soxhlet apparatus (Method I) or dichloromethane solution of animal or vegetable fat was passed through the 1g of active carbon placed in 8 mm i.d. glass column. After refluxing the column with dichloromethane within 2 hours followed by toluene within 30 minutes the column was reversed upside down and refluxed with fresh portion of toluene for 16 hours accordingly to the procedure described in ².

After solvent evaporation the residue was dissolved in hexane and further purified as for method I.

Analysis:

Analysis was performed using ThermoQuest GCQplus GC-MS/MS systems adjusted to double fragmentation mode. In this case, secondary ions which are formed by splitting out of COCl particle from PCDD/PCDF molecular ion M^+ resulting with $(\text{M}-\text{COCl})^+$ ion were detected. For non-ortho-PCBs $(\text{M}-\text{Cl})^+$ secondary ions were detected respectively. Gas chromatograph conditions are as follows: helium flow set to 1.0 ml/min. DB-5MS or DB-17 Column temperature programs are as follows: 100°C for 1 minute, $20^{\circ}\text{C}/\text{min}$ to 200°C , $2^{\circ}\text{C}/\text{min}$ to 280°C and hold for 15 minutes. Transfer line temperature was adjusted to 270°C . Because of different structures of cores of PCDDs and PCDFs molecules, optimum of collision energies with helium atoms in a MS/MS system differ.

Methods validation:

The described procedure was validated using "dioxin free" sunflower oil sample obtained after dioxin removal by passing 100 ml of 20%v/v oil dichloromethane solution through the active carbon column and further solvent evaporation.

Each of the 5g portions of the sample were spiked with a mixture of the 17 of $^{12}\text{C}_{12}$ -2,3,7,8-congeners and the 3 of non-ortho $^{12}\text{C}_{12}$ -PCBs. In this case precision is lower than 10% for 2,3,7,8-TCDD and TCDF. Relative standard deviations for single PCB measurement was better 15%. Mean values were calculated on the result of twelve independently performed analyses of the standard sample of the oil in the same conditions, using the same batch of solvents and chemicals.

Analyses were carried out in the same conditions as for real samples using the GC-MS/MS system equipped with autosampler.

Results and Discussion:

In comparison to the high resolution mass spectrometric systems the use of quadruple double fragmentation MS/MS detection also can be used successfully in the determination of dioxins in biological samples. It was also reported^{3,4,5} that double fragmentation mass spectrometric detector is a powerful tool for ultra trace analysis and in many cases may substitute high-resolution mass spectrometric systems.

The results show that the presented method is very efficient for dioxin analysis

So far more than 400 food samples have been successfully analysed with the described method. GC-MS/MS method has achieved limit of detection of 0,1 pg/g of fat for 2,3,7,8-TCDD and 0,2 pg/g of fat for 1,2,3,7,8-P₅CDD. For animal fat samples a typical PCDD/F congener distribution was observed.

Very specific PCDD and PCDF congener distribution was found for Baltic fish and fish oil samples. In figure 1 it is shown that 2,3,7,8-TCDF is the most abundant congener among the other TCDFs. Similar correlation was found for 2,3,7,8-TCDD and the other 2,3,7,8-chlorosubstituted dioxins and furans in fish samples.

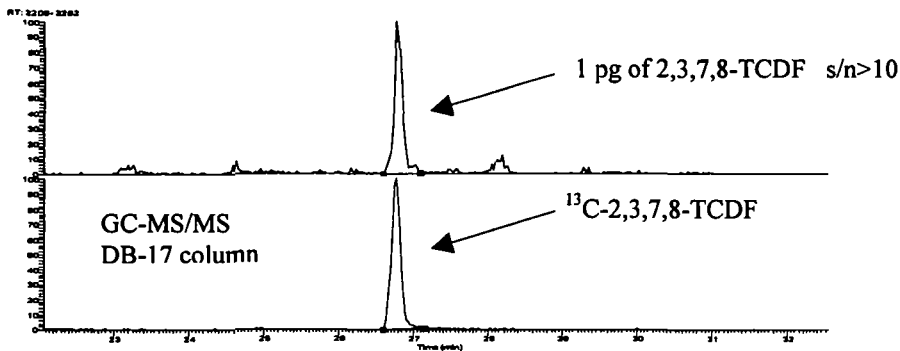


Fig. 1. Typical MS/MS chromatogram of TCDF fraction in fish fat from Baltic Sea (Gdansk Bay) sample

The highest relative concentration of the 2,3,7,8-TCDF was found in the most of the Baltic fish samples.

Very similar 2,3,7,8-chlorosubstituted congener contribution were also found in P₅CDDs/Fs groups as well as in H₆CDDs/Fs. The elevated concentration of 2,3,7,8-chlorosubstituted PCDDs and PCDFs congeners is probably caused by higher bioaccumulation of these compounds in fish adipose tissue.

Result of the determination of PCDDs/PCDFs and coplanar PCBs in about 400 of samples of Polish food is presentse in table 1.

Table 1: The result of the analysis of samples of Polish food in 1999

Food Product	Dioxin concentration in ng-TEQ/kg fat
Milk	0,1 – 4,0
Powdered milk	0,3 – 5,0
Butter	0,6 – 6,5
Yellow cheese	0,2 – 7,7
Yoghurt 2 - 5% of fat	0,1 – 1,8
Pork	0,05 – 1,3
Beef	2,4 – 8,5
Grilled beef on open fire	20 - 25
Poultry	0,6 – 12,8
Polish fishes from river waters	1,2 – 9,4
Baltic Sea fishes	7,0 – 40,0
Fish oil from Baltic Sea fishes	11,2 - 50
Eggs (boiled)	0,6 – 7,4
Fresh vegetable oil	0,02 – 0,1
Fried used vegetable oil	0,15 – 0,8
Fishmeal	6,5 - 20
Animal meal	0,25 – 4,25
Chocolate products	0,05 – 0,75

As it is presented in table 1 for grilled meat elevated dioxin concentration was observed. The samples of grilled meat were taken from the market in Krakow centre.

In this case the meet was grilled on an open fire.

In most of the samples all of the seventeen PCDDs and PCDFs congeners were detected as well as the three coplanar non-ortho PCBs.

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