PTV-LV INJECTION COUPLED TO CAPILLARY GC ION TRAP MS/MS AS ALTERNATIVE METHOD FOR PCDD/Fs ANALYSIS IN FOODS.

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Introduction

Since the PCB's crisis during spring 1999, dioxins and PCB's monitoring program of the food chain took place in Belgium.
Authority guidelines for animal feed and food products monitoring of persistent pollutants such as PCDD/Fs are to support laboratories to develop and validate screening methods.
GC-MS/MS as screening method to analyze dioxins and furans in different matrices such as fly ash, soils, sediments, fish, and human milk has been widely described in the literature[1,2,3,4,5]. But dioxin levels in foods are usually sub-parts-per-trillion, and practical limits of quantification using great amount of sample (i.e. 30 to 100 g fat) by MS/MS have been measured[6]. From that point of view, sensitivity has to be considered as a primary parameter to carry out this type of analysis. New generation of ion trap mass spectrometer in MS/MS mode compared to present high-resolution mass spectrometer technology in SIM mode, provides for 2,3,7,8 TCDD lower sensitivity by a factor 5 to 10.
To achieve lower method detection limit (MDL), there are two possibilities. One is to increase the sample size; the other one is, if possible, for the same sample size, to increase the amount of analyte injected. The first possibility doesn't make sense regards to screening method. The second one has been investigated. Large volume injection has been selected in order to increase the method sensitivity and to reduce the evaporation time before analysis.
Limits of quantification of PTVLV-GC-MS/MS technique for PCDD/Fs in different food matrices, using at once the same sample size and the same sample preparation as for GC/HRMS injections, have been evaluated.

Methods and Materials

Sample preparation
10 g of certified spray dried milk powders (BCR 607, RM 534, RM 533, IRMM, Geel, Belgium) were mixed and homogenized (1:1:1:1) with water (HPLC, Riedel de Haen, Seelze, Germany), sodium sulphate (J.T. Baker, Deventer, holland) and silicagel (Merck, Darmstadt, germany) before extraction. Sixteen hours Sohxlet extraction using dichloromethane/pentane (1:1) (Riedel de Haen, Seelze, Germany) was performed. Lipids content (3 g of fat) were determined gravimetrically after extraction and spiked with the 17 PCDD/PCDFs ^{13}C labeled compounds (CIL, Andover, MA, USA).
4 g aliquot of our internal QC (500 g of beef fat was fortified with native PCDD/Fs at 0.4 pg/g for TCDD/F, 2 pg/g for Penta- to HepaCDD/F and 4 pg/g for OCDD/F to have a total content of approximately 5 pg TEQ/g fat) were directly processed on automated clean-up step.

Automated dioxins clean-up for food analysis has been widely described[7]. The procedure can be summarized as follow: Approximately 3-4 g of extracted fat diluted in hexane (ACROS, Geel, Belgium) are directly loaded on High Capacity Disposable Silica columns (HCDS) connected to multilayer silica, basic alumina and PX-21 carbon columns (FMS inc, Watertorn, MA, USA). Samples are running through the columns on an automated Power-Prep System™ (FMS inc, Watertorn, MA, USA). All solvent used were pestanal quality (ACROS, Geel, Belgium). The final extract in toluene is evaporated until 30 µL and completely injected in GC.

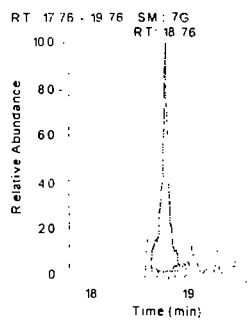
Analysis

All ion trap MS/MS analysis were conducted on a ThermoQuest Trace GC PolarisQ ion trap mass spectrometer (Austin, Tx, USA) equipped with a Rtx 5-MS (30m x 0.25 mm x 0.25 µm) capillary column (Restek, Evry, France). Programmable Temperature Vaporization-Large Volume (PTV-LV) injections were carried out using a Combi Pal Autosampler (CTC analytics, AG, Zwingen, Switzerland) and a Silcosteel deactivated liners with silica whool purchased from ThermoQuest (Italy).

Results and discussion

Before large volume injection optimization, sensitivity performances of bench top PolarisQ ion trap MS and high resolution MS (MAT 95 XL and Ultima Micromass) have been compared.

500 fg of TCDD injected in splitless mode and detected with a S/N ≥ 3 is normally achieved by ion trap MS/MS. By installing new hardware and software on PolarisQ that increase the helium damping gas pressure inside the ion trap, this will result in an increase in trapping efficiency allowing for lower detection limits[8].



As shown in figure 1, 200 fg of TCDD has been detected with S/N ≥ 5. Note that higher helium pressure in the trap induces higher excitation voltage (i.e. 5 to 6 V).

Comparatively, 40 fg of TCDD can be routinely detected by HRMS with S/N ≥ 10 proving that HRMS is 5 to 10 times more sensitive.

Figure 1 : Detection of 200 fg of TCDD by ion trap MS/MS technique.

Optimization of PTVLV-GC/MS/MS was achieved by using PCDD/Fs standard solutions diluted in toluene. The solvent choice is extremely important due to the fact that PTVLV injections allow large volume injections when the sample components are less volatile than the solvent. For example, PTVLV injections for less chlorinated PCBs in toluene is not advisable.

Programmable temperature injection mode can be divided into 4 phases: injection, vaporization, transfer and cleaning. Main parameters optimized are summarized in Table 1.

PTVLV method	
Base temperature	100°C
Injection time	0.1 min
Split flow	100 ml/min
Evaporation temperature	120°C
Evaporation rate	14.5°C/sec
Evaporation time	0.5 min
Transfer temperature	300°C
Transfer rate	14.5°C/sec
Transfer time	1 min
Cleaning temperature	300°C
Cleaning time	30 min
Cleaning flow	100 ml/min

30µL of liquid sample in toluene enter the injector in "cold conditions" (100°C), then solvent is vaporized by rapidly increasing the temperature to 120°C. During these two steps, the splitting valve is open. In sample transfer, the temperature raise rapidly to 300°C and the splitting valve is closed for 1 minute. Liner can be cleaned during GC run by opening the splitting valve and let the liner temperature at 300°C.

Table 1: PTVLV injection parameters optimized for PCDD/Fs analysis.

The final volume (i.e. 30 µL) is a good compromise between the amount of liquid injected in the liner and the time saved during the evaporation step before analysis. Nevertheless, we found that after several injections, chromatographic resolution goes down and a pre-column has to be installed. An other problem encountered is that toluene is still remaining in the trap several hours after injection. One solution to solve the problem is to install a back-flush system to purge with helium the pre-column and the liner during the GC run.

Beef fat fortified in the range of 0.4 to 4 pg/g fat from tetra-to-octaCDD/Fs provide accurate results excepted for TCDF where in interference co-elute (figure 2). Mean values calculated in triplicate by PTVLV-GC/MS/MS have been compared to mean values (n=10) obtained by GC/HRMS. RSDs are in the range of 10 to 20 % that can be considered as satisfactory at parts-per-trillion levels.

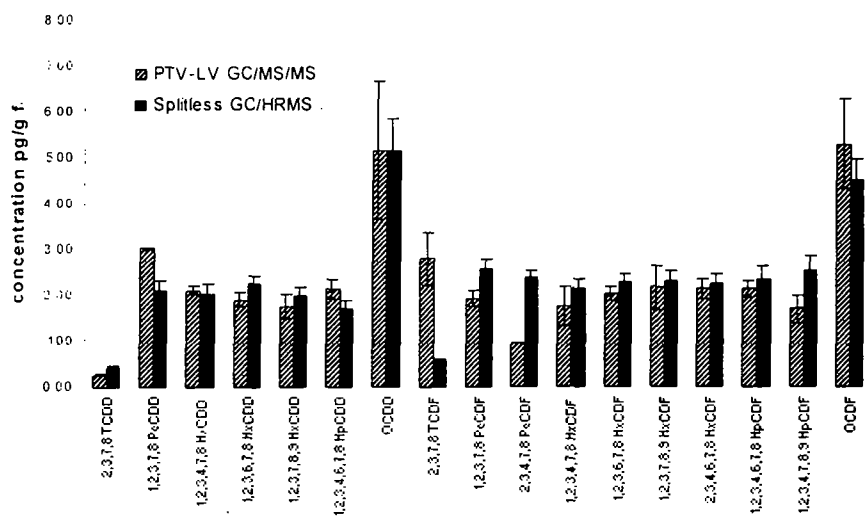


Figure 2 : PTVLV-GC/MS/MS and GC/HRMS comparison for fortified beef fat.

Trueness of the method has been tested using 10 g of different reference materials (RM534, RM533) and certified material (BCR 607) spray dried milk powder.

BCR 607	Calculated value	Certified value	Uncertainty	Trueness
Compounds	pg/g	pg/g	pg/g	(%)
2,3,7,8 TCDD	0.24	0.25	± 0.03	96
1,2,3,7,8 PeCDD	0.74	0.79	± 0.04	94
1,2,3,4,7,8 HxCDD	0.43	0.42	± 0.07	102
1,2,3,6,7,8 HxCDD	1.00	0.98	± 0.11	102
1,2,3,7,8,9 HxCDD	0.30	0.34	± 0.05	88
2,3,7,8 TCDF[a]	nd	0.05	± 0.03	
1,2,3,7,8 PeCDF[a]	nd	0.05	± 0.01	
2,3,4,7,8 PeCDF	Interference	1.81	± 0.13	
1,2,3,4,7,8 HxCDF	0.83	0.94	± 0.04	88
1,2,3,6,7,8 HxCDF	1.06	1.01	± 0.09	105
1,2,3,7,8,9 HxCDF				
2,3,4,6,7,8 HxCDF	1.12	1.07	± 0.05	105

a : Value near or at the determination limit

Table 2 summarized results obtained for the BCR 607.
Trueness expressed as bias (%) is in the range of 88 to 105 % for most of the congeners except for TCDF and PeCDF. In fact, 10 g test portions is not enough to detect them even by GC/ HRMS.

Table 2 : Trueness of PTVLV-GC/MS/MS method on BCR 607.

An interference at the retention time of 2,3,4,7,8 PeCDF was present and probably due to several large volume injections in the column. Ruggedness has still to be evaluated. But, a pre-column equipped with a back-flush system would solve the problem and would increase life time of GC columns. Figure 3 shows chromatograms of TCDD and HxCDF from BCR 607 sample proving that limit of quantification between 0.1 and 0.2 pg/g fat for all congeners would be achieved.

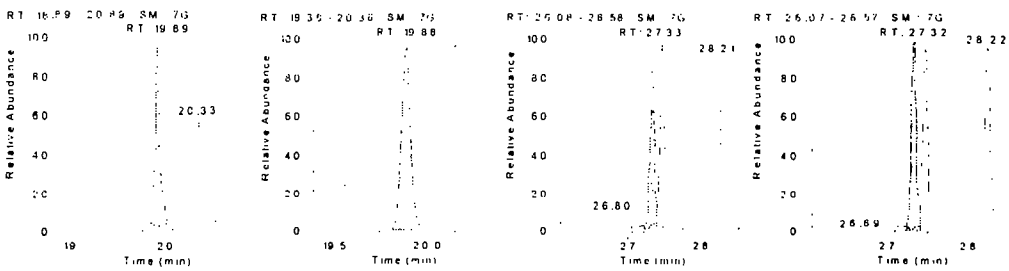


Figure 3: Chromatograms of TCDD native, ^{13}C and HxCDF native, ^{13}C from BCR 607.

Acknowledgements
The authors wish to thank J. Ragsdale from ThermoQuest (Austin, USA) and Interscience (Belgium) for technical support.

References

1. J.F. Focant, G. Eppe , E. De Pauw, *Chemosphere*, 43, 2001, 417-424
2. Y. Tondeur, W.N Niederhut, J.E Campana, *Biomed. Environ. Mass Spectrom*, 1987, 14, 449
3. M.J Charles, W.C. Green, G.D Marbury, *Environ. Sci. Technol.*,1995, 29, 1741
4. E.J.Reiner, D.H. Schellenberg, V.Y Taguchi, *Environ. Sci. Technol.*,1991, 25, 110
5. A.P.J.M De Jong, A.K.D. Liem, A.C. den Boer, E. van der Heeft, J.A. Marsman, G. Van de werken, R.C.C Wegmen, *Chemosphere*, 1989, 19, 59
6. D.G. Hayward, K. Hooper and D. Andrzejewski, *Anal.Chem.*,1999, 71,,212-220
7. J.F. Focant, G. Eppe , E. De Pauw, *Organohalogen compounds*, 2000, 45, 49-52
8. J.D. Ragsdale, T.M. Harvey, Pittcon 2001