Comprehensive multidimensional gas chromatography coupled to low resolution quadrupole mass spectrometry for the analysis of PCDDs, PCDFs and PCBs

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Introduction

Because of the high persistency and extreme toxicity of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and so-called dioxin-like polychlorinated biphenyls (PCBs), their trace level determination is a topic of much interest [1]. The typical concentrations of this compounds, sub-ng/kg, makes that they have to be clearly separated from other, less toxic, congeners present in the samples and from the matrix and the use of sensitive techniques is required for the quantification.

The analyses of the compounds are usually done by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS). Recently, alternative novel techniques have been developed which are improving the chromatographic separation, e.g. comprehensive multidimensional gas chromatography (GC×GC), for the analysis of the compounds [2]. The aim of this work is to evaluate GC×GC coupled to a low resolution quadrupole mass spectrometer for the quantification of PCDDs, PCDFs and dioxin like PCBs.

Materials and methods

Samples and chemicals: A standard solution containing seventeen 2,3,7,8-substituted CDD/F congeners and twelve dioxin-like CBs was prepared by mixing two commercial standard mixtures, EPA 8290 STN and WP-STK, both from Wellington (Guelph, ONT, Canada) in n-nonane. A ten-fold-diluted mixture was used to optimize the GC×GC-MS conditions. Individual standards of CBs and CDD/Fs used for peak identification were purchased from Promochem (Wesel, Germany) and Cambridge Isotope Labs (Andover, MA, USA), respectively.

A milk sample was prepared as follows. Two hundred gram of milk, purchased in a supermarket (Barcelona, Spain), was spiked with 10 pg of TCDD/F, 50 pg of Pe-, Hx- and HpCDD/Fs and 100 pg of OCDD/F. The sample was extracted with potassium oxalate (15%), ethanol, ethylic ether and hexane, and purified on a silica column (anhydrous sodium sulphate, sulphuric acid-silica, glass wool), and then on a multilayer silica column (anhydrous sodium

sulphate, sulphuric acid-silica, activated silica, sodium hydroxide-silica, activated silica, silver nitrate-silica, glass wool). The purified extract was fractionated using a SPE carbon tube. Two fractions were collected: the first one, containing CBs and the second one containing CDDs/Fs. The CB fraction was further fractionated on a HPLC pyrenyl column to separate dioxin-like CBs from the bulk CBs. Both fractions of interest – dioxin-like CB and CDD/F – were finally purified on a silica column (anhydrous sodium sulphate, activated silica, sulphuric acid/silica, glass wool) and concentrated to 25µl [3].

Instrumentation: All experiments were performed using a Clarus 500 MS system (Perkin-Elmer, Shelton, CT, USA). Turbo mass software was used for control, general operation and acquisition of the MS. For data transformation, evaluation and visualization of the GC×GC chromatograms ChromCard software (ThermoFinnigan, Milan, Italy) was used. Transform software (Fortner Research, Sterling, VA, USA) was used for producing 2D chromatograms. The modulator used was a two jet modulator using liquid CO_2 as a cooling gas. The modulator period was 8s with a modulation time of 4s in each jet. A 30m x 0.25mm x 0.25µm VF-1ms (100%-dimethylpolysiloxane) purchased from Varian (Middelburg, the Netherlands) and a 0.5m x 0.18mm x 0.1µm LC-50 (50%-liquid crystalline-methylpolysiloxane) purchased from J&K Environmental (Sydney, Nova Scotia, Canada) were used as the first- and second-dimension columns, respectively. The columns were connected by means of a press-fit connector (Techrom, Netherlands). The initial oven temperature was 90°C (2min), followed by a temperature ramp of 30°C/min to 170°C (5 min.), then at 1.5°C/min to 270°C (10 min). Helium (Hoek Loos) with a purity of 99.999% was used as carrier gas though the GC×GC system at a constant flow of 1.2 ml/min. Injector temperature was maintained at 280 °C and the splitless injection mode (2 min) was used.

The ion source temperature for electron impact (EI) was set at 140°C for PCBs and PCDFs, and at 170°C for PCDDs. The transfer-line temperature was 280°C. For EI mode, the electron energy was 70eV, the emission current was 100 µA, multiplier voltage was set at 400V and the lens L1 and L2 were tuned at 10V and 190V, respectively. For the NCI mode, the electron energy was 50eV, the emission current 190 µA, multiplier voltage was set at 600V and the lens L1 and L2 were tuned at 190V and 10V, respectively.

In both ionizations modes, full scan data acquisition was performed over a mass range of 250 amu, 150 amu and 25 amu. To evaluate the quality of the peaks with the three mass ranges the limits of detection (LODs) and the repeatability (RSD) were determined. The LOD was calculated as the amount injected and a signal to noise ratio (S/N) of 3. The RSD was determined based on five replicate injections of the standard solution. For the NCI mode the ion source temperature (150°C and 200°C), and the methane pressure (5.5e-5 to 1.0e-4 Torr) were optimized.

Results and discussion

Optimization of GC×GC-EI-MS: Using GC×GC a high scan speed of the MS is necessary (minimum circa 40Hz) to detect the peaks. To obtain a high scan speed, a constant number of data points per peak, and a mass range that was in the area of the compounds, the MS had to be recalibrated to a scan speed of 16000 Da/s. Calibration was performed from m/z 200 to 500 at a low velocity of **0.4 s and at a high velocity of 0.024 s.** In general, the best results were obtained with the small mass range (25 amu). The quality of the spectra and peak shape was higher, due to the fact that with a small mass range an average of two or more spectra per data point can be

obtained. As expected the lowest LODs were observed for the 25 amu range, see table 1. LODs varied between Xpg and Xpg for the CBs, and from Xpg to Xpg for the PCDDs/Fs. For the repeatability a relationship between the scan speed of the MS and mass range was found. The repeatability improved from the 250 amu to the 25 amu range; 29% and 41% for the 250 amu range, 10% and 22% for the 150 amu range, and from 5% to 9% for the 25 amu range.

Optimization of GC×GC-NCI-MS: The same calibration procedure of the MS as for EI mode was used for NCI mode. The optimum ion source temperature was found to be 150°C for the dioxin-like CBs and PCDFs, and 170°C for PCDDs, and the optimum methane pressure was 7.5e-5 Torr.
As an illustration of the peak shape, number of scans in a peak, and MS spectra, figure 1 shows the results for CB 77 and CB 81. A large improvement in the peak shape and quality of the spectra was found from the 250 amu to the 25 amu range. In general, the LODs for PCBs, PCDFs and PCDDs in NCI mode were lower than the LODs for EI mode, see table 1. The exceptions are the lower chlorinated compounds (tetra- and some penta-chloro congeners), which is in agreement with results of other studies [3, 4]. For the hexa- and heptachlorinated congeners the LODs are ten times lower in NCI than in EI mode. The LOD for all compounds are higher than for GC×GC coupled to the µ-ECD, with the exception of CB169 and CB189. In general, the LODs for PCDFs were lower than for PCDDs.

Table 1: Limits of detection (pg) for dioxin-like CBs for GC×GC coupled to low resolution MS in EI and NCI mode, and for GC×GC coupled to µ-ECD. LODs for three mass ranges (250 amu, 150 amu and 25 amu) are shown. The relative standard deviation (RSD) is given in brackets.

Compound	250 amu		150 amu		25 amu		
	EI	NCI	EI	NCI	EI	NCI	µ-ECD
81	15 (34)	2.8 (28)	7 (10)	2.3 (14)	0.9 (5)	0.9(10)	0.06
77	21 (32)	3.6 (28)	10 (11)	1.7 (13)	1.0 (6)	1.1(11)	0.07
123	28 (32)	3.7 (9)	6 (10)	1.6 (9)	1.2 (8)	0.9(11)	0.04
118	15 (31)	1.7 (11)	5 (11)	1.4 (15)	1.35(8)	0.7 (10)	0.04
114	7 (31)	0.7 (11)	2 (15)	0.4 (11)	0.86(6)	0.3 (8)	0.03
105	30 (29)	2.5 (12)	7 (16)	2.1 (10)	1.17(5)	1.0 (10)	0.03
126	69 (32)	0.4 (11)	12 (22)	0.3 (16)	2.16(7)	0.2 (6)	0.06
167	22 (39)	0.3 (12)	11 (18)	0.4 (16)	0.92(6)	0.1 (10)	0.05
156	61 (35)	0.6 (12)	16 (18)	0.4 (12)	1.45(5)	0.2 (13)	0.03
157	38 (35)	0.5 (50)	16 (17)	0.8 (14)	2.78(5)	0.3 (9)	0.04
169	83 (41)	0.4 (8)	19 (13)	0.2 (11)	1.12(8)	0.08(12)	0.05
189	42 (37)	0.2 (18)	17 (17)	0.2 (24)	1.85(9)	0.09(8)	0.04

Application: The spiked milk sample was analyzed with GC×GC-NCI-MS using the optimum conditions. The results of GC×GC-NCI, GC×GC-µECD and HRGC-HRMS are given in table 2. As can be seen, the results for CBs, PCDDs and PCDFs are similar to the GC×GC-µECD and HRGC-HRMS. For some CBs lower levels were found with GC×GC-NCI-MS than with GC×GC-µECD and HRGC-HRMS, which indicates that interfering compounds were present by the later two

techniques. On the other hand, for some CBs (CB77, CB 156 and CB169) higher levels were observed for GC×GC-NCI-MS compared to the other techniques. The main disadvantage of the GC×GC-NCI-MS system is limited sensitivity for PCDDs and PCDFs. The spiking level of the milk sample was close to the detection limit for tetrachloro- and pentachloroCDDs and -CDFs.

Conclusions

- The GCxGC coupled to low resolution mass spectrometry was studied for the analysis of PCDDs, PCDFs and dioxin-like CBs. The EI mode and NCI mode was tested. The optimum source temperature was 140°C for PCDFs and CBs, and 170°C for PCDDs, and the optimum NCI gas pressure was 7.5e-5 Torr.
- The LODs and repeatability of the system was acceptable for a small mass range (50 to 25 amu).
- Finally, the results show that the method is suitable for the analysis of dioxin-like CBs, but less suitable for the analysis of low levels of PC DD/Fs, which is a result of the low sensitivity of the low resolution mass spectrometer for these compounds.

Table 2. Levels (pg/X) PCDDs/Fs and dioxin-like PCBs in a spiked milk sample spiked for GC×GC-MS, GC×GC-µECD, and GC-HRMS.

Compound	GC×GC-NCI	µ-ECD	HRMS	compound	GC×GC-NCI	µ-ECD	HRMS
4f1	10	20	19	81	3.0	3.8	4.2
4d1	46	<dl	22	77	11	4.5	3.5
5f1	12	18	20	123	5.9	22	20
5f2	35	36	41	118	5.6	23	21
5d1	28	<dl	35	114	50	22	18
6f1	4.4	3.3	2.8	105	21	17	51
6f2	17	8.9	44	126	12	26	20
6f3	558	56	104	167	10	2.5	19
6d1	3249	3700	3067	156	43	18	17
6d2	178	67	73	157	19	23	19
6d3	1834	1100	1268	169	81	16	19
6f4	28	35	31	189	12	23	19
7f1	146	190	193				
7d1	310	410	428				
7f2	92	89	101				
8d	5.4	6.5	7.7				
8f	50	37	41				

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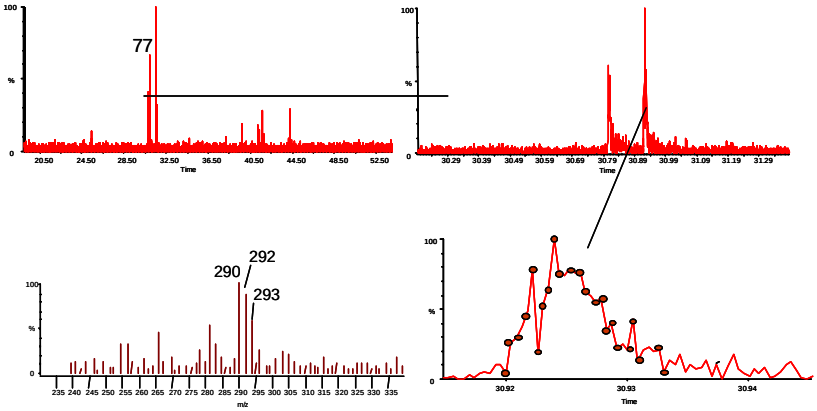
References

1. Schecter A., Pavuk M., Malisch R., Ryan J.J. (2003) J. Toxicol. Environ. Health A 65, 1391.
2. Korytár P., Danielsson C., Leonards P.E.G., Haglund P., de Boer J., Brinkman U.A.Th. (2004) J. Chromato. A, 1038, 189.
3. Bagheri, H., Leonards, P.E.G., Ghijsen, R.T., Brinkman, U.A.Th. (1993). Intern. J. Environ. Anal. Chem. 50 (4), 257.
4. Rothweiler, B, Berset J-D. (1999). Chemosphere 38 (7), 1517.

A



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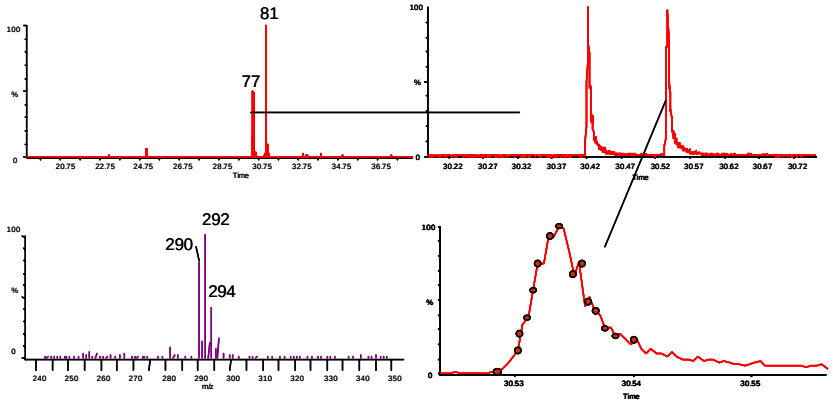

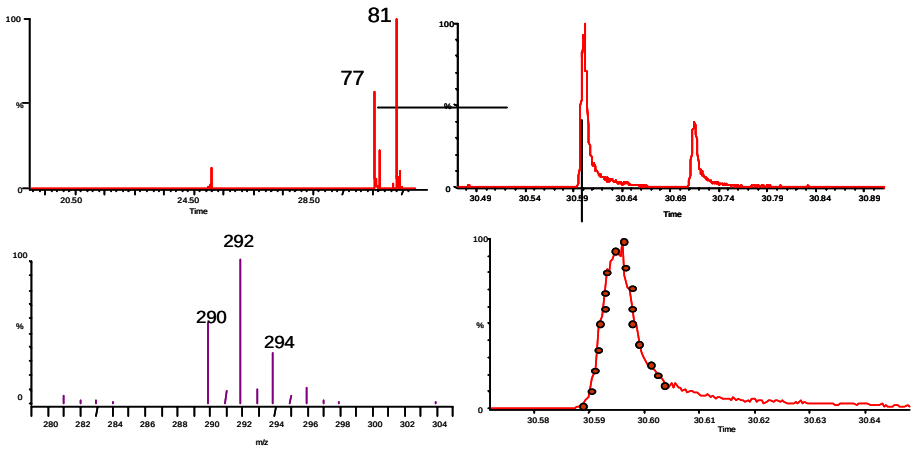

Figure 1. GC×GC-NCI-MS chromatograms for CB 77 and CB 81. MS spectra obtained and the number of data points per peak at A) 250 amu mass range, B) 150 amu mass range, C) 25 amu mass range.