3 Development of a programmed temperature vaporizer-large volume injection-gas chromatography-ion trap MS/MS (PTV-LVI -GC-Ion trap MS/MS) method for dioxins and furans in food and feed

3.1 Summary

The potential of a PTV-LVI-GC-ion trap MS/MS method as an alternative method for the measurement of PCDD/Fs in food and feed is reviewed. The sensitivities of ion trap MS/MS instruments are typically less than HRMS, but with slight adjustments to MS/MS parameters, sample size and final extract volume, GC/HRMS quality control specifications can be achieved. The main MS/MS parameters were optimized with an emphasis on the use of damping gas pressure inside the ion trap to achieve the highest sensitivity. An instrument detection limit (IDL) of 200 fg μL^{-1} injected with a signal-to noise ratio of 5:1 for TCDD was obtained. In addition, the optimisation of a programmed temperature vaporizer (PTV) method allows a larger sample volume injection, essential for the detection and the quantification of trace levels of PCDD/Fs in biological samples. An injection volume of 10µL was found to be the best compromise between the sensitivity requirements and the robustness required for large number of injections. Five matrices (Beef fat, eggs yolk, animal compound feed, milk powder and serum samples), covering a concentration range of two orders of magnitudes at the parts-per-trillion levels, were used to validate the alternative method. An analysis of variance (ANOVA) was carried out. In toxic equivalence (TEQ), the study proved that no significant bias was observed between the alternative and the reference methods even if three toxic congeners showed significant p-values. The potential of the method was then evaluated in the framework of a European project which concerned a feasibility study on five candidates' matrix CRMs in food and feed. Results showed a small positive bias proportional to concentration levels. However, the method complied with the trueness criteria of EU Commission Regulation 1883/2006.

3.2 MS/MS principles for dioxins

Dioxin example in MS/MS mode by ion trap mass spectrometry, used in the framework of analytical purposes, is a wonderful application and illustration of the theory presented in chapter 2. The lack of selectivity due to the unit mass resolution is compensated by operating the instrument in MS/MS mode (Focant et al., 2005). This is referred to as tandem-in-time mass spectrometry because the process takes place in three successive steps (as mentioned in paragraph 2.6.2.5): (1) selected parent ions are isolated in the trap after ionisation, (2) their dissociation by CID and (3) the product ions are sequentially ejected from the trap by ramping the rf voltage and further detected by an electron multiplier. The ionisation by electronic impact (EI) can either occur in an internal source inside the trap (VARIAN configuration) or in an external source with further acceleration of the produced ions through lenses and their introduction into the ion trap analyzer (Thermo configuration). A global overview of MS/MS scan functions occurring in-time is shown on the Figure 3-1 for the TCDD example (March et al., 1997). The abscissa axis represents the time in milliseconds and the ordinate represents the amplitude of the voltages. RF corresponds to the potential applied to the ring electrode and the supplementary alternating voltages applied to the end-cap electrodes in dipolar fashion, which are referred to as waveforms. The first step consists of isolating the two most intense ions in the molecular ion cluster (e.g. m/z of 320 and 322), that is the predominant transition $[M]^{+\bullet}$ and $[M+2]^{+\bullet}$. Ions with m/z < 320 are ejected using the mass selective axial instability mode by ramping the rf amplitude voltage. The ions' ejection is facilitated by the concurrent application of axial modulation with an amplitude of 3V. When ions of m/z 320 arrive close to the instability region (q_z = 0.908), the rf amplitude is modulated moderately in order to avoid the ejection of the selected ions. Then, when ions of m/z <320 are ejected, the rf is a little bit decreased and the ejection of ions with m/z > 322 can start. It is achieved by applying a broadband waveform. Ions are ejected by matching the frequency (500 Hz steps) with the secular frequency of ions of a higher m/z ratio. Once isolation of the selected ions is completed, the rf voltage is dropped to obtain a q_z value of 0.4. Ions of m/z 322 migrate on the left side of the axial q_z axis to a more stable region of the diagram. By using equation (2-23), the LMCO is equal to m/z 142. In MS/MS, CID process can be affected in four modes: (1) single frequency irradiation (SFI), (2) multi-frequency irradiation (MFI), (3) secular-frequency modulation and (4) non-resonant excitation (Wang et al., 1996). The first three resonant modes were investigated and compared for dioxin application (Plomley and March, 1996). They

concluded that the tuning requirements of MFI and the duration of irradiation were compatible with the gas chromatographic time scale.

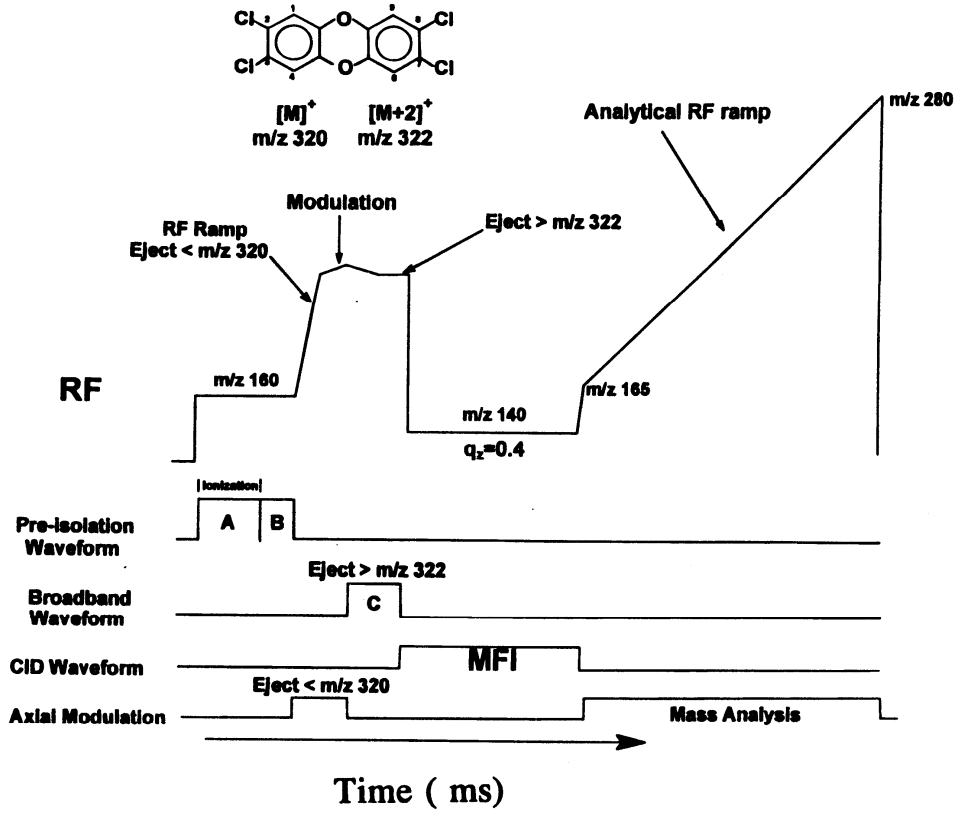

Figure 3-1 : Scan function for MS/MS of dioxin (TCDD)

During CID process, PCDDs fragmentation is characterized by losses of $Cl^{\bullet}$, $COCl^{\bullet}$, $2COCl^{\bullet}$ and $Cl^{\bullet}$, $COCl^{\bullet}$, $COCl_2$ and $COCl_3^{\bullet}$ for PCDFs. The main fragment used for quantification by isotopic dilution technique is the loss of $COCl^{\bullet}$ for both PCDDs and PCDFs (Figure 3-2) while the loss of Cl_2 characterizes the main fragment used for PCBs quantification.
Following CID by MFI for 30 ms, the analytical mass range of 165-350 Da is scanned for product ions by ramping the rf voltage.

Native

$[C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2]^{+\bullet}$ $\rightarrow [C_{11}H_4{}^{35}Cl_2{}^{37}ClO]^{+} + CO^{37}Cl^{\bullet}$

(m/z = 322) (m/z = 257)

$\rightarrow [C_{11}H_4{}^{35}Cl_3O]^{+} + CO^{35}Cl^{\bullet}$

(m/z = 259)

Labelled

$[^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2]^{+\bullet}$ $\rightarrow [^{13}C_{11}H_4{}^{35}Cl_2{}^{37}ClO]^+ + {}^{13}CO^{37}Cl^{\bullet}$

(m/z = 334) (m/z = 268)

$\rightarrow [^{13}C_{11}H_4{}^{35}Cl_3O]^+ + {}^{13}CO^{35}Cl^{\bullet}$

(m/z = 270)

Figure 3-2 : Product ions from native and labelled $^{13}C_{12}$ TCCD using ion trap MS/MS

3.3 Isotopic dilution technique for quantification by MS/MS

The principle is exactly the same as already reported for HRMS (see paragraph 2.6.1.4). The main difference is characterized here by the fact that instead of following the two most abundant molecular ions at 10000 resolution in SIM mode, the two product ions [M+2-$CO^{35}Cl^{\bullet}$] and [M+2-$CO^{37}Cl^{\bullet}$] are monitored for both native and labelled molecular ions (see Figure 3-2). It is called a multiple reaction monitoring (MRM). Within a time window, the instrument alternatively scans the native and the label congener. Only 2,3,7,8-chloro-substituted congeners are followed. As for HRMS, the chromatogram is sliced into seven time windows from tetra-through-octa chlorinated dioxin and furans on a Rtx-5MS as shown on Figure 3-3 and in Table 3-1. Peaks are detected when a signal to noise ratio (S/N) is ≥ 3. Compounds are numbered from 1 to 17 from TCDF to OCDF (see Table 3-1) for numbering correspondences). As native congeners coelute with its corresponding labelled $^{13}C_{12}$ isomer, the native peak maxima should fall within 3 seconds of their corresponding ^{13}C labelled analogues for identification.

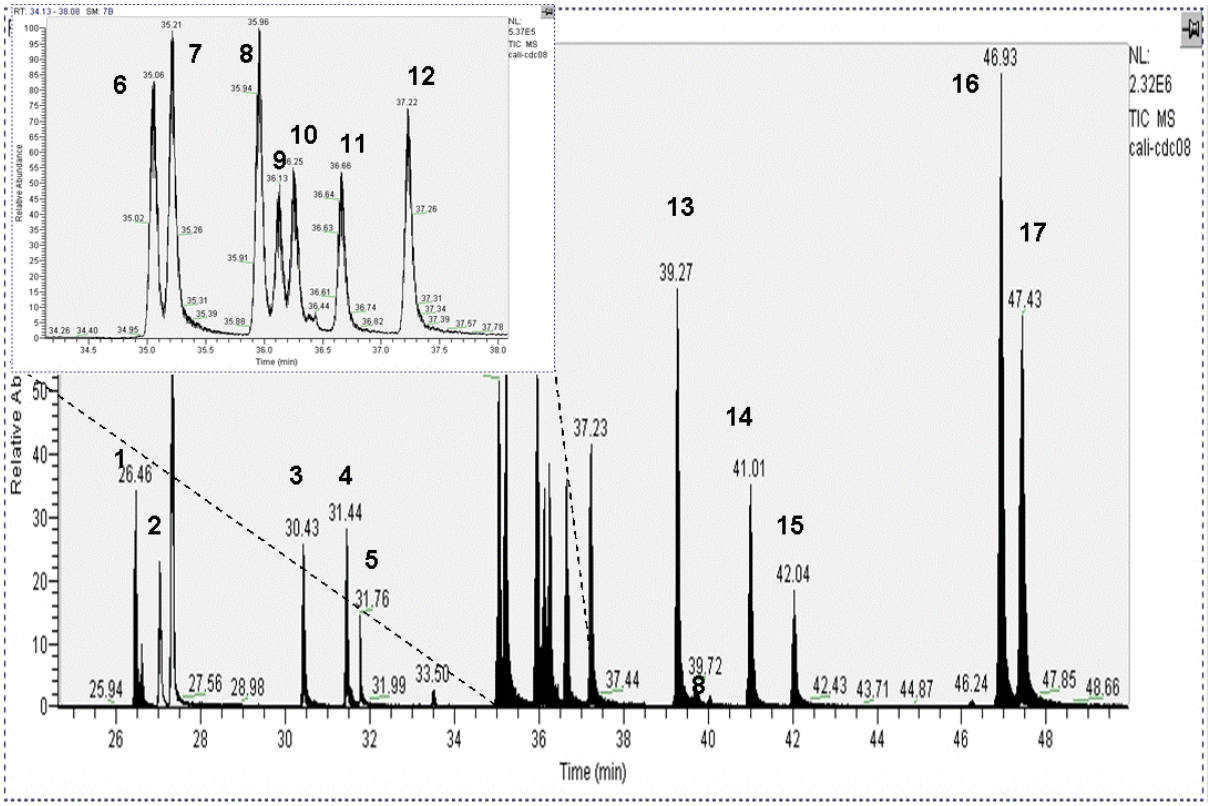

Figure 3-3 : Retention time of the seven toxic PCDDs and ten PCDFs on a Rtx5-MS column

In windows 1, 2 and 4, the native compound and its corresponding labelled internal standard are monitored in MRM mode. For windows 3, 5, 6, and 7, MRM is performed by monitoring alternatively four molecular ions. After isolation, the molecular ion is replaced in the stability diagram at a value of $q_z = 0.45$. The optimum voltage applied to the end-cap electrodes during CID varies between 5.5 and 6 Volts while CID time has been optimized to 30 ms.

The specificity of MS/MS is achieved by monitoring two product ions and by checking their isotopic ratios. However, the isotopic contribution of the fragment ions depends not on the natural distribution of their constitutive elements but on the selection of the precursor. Indeed, as shown on Figure 3-2, there is one chance out of four to lose $CO^{37}Cl^{\bullet}$ while there are three chances out of four to lose $CO^{35}Cl^{\bullet}$. Thus, the ratio of product ions (i.e. the ratio 257/259) equals to 0.33. The others ratios from tetra to octa chlorinated congeners can be calculated the same way. Obviously, to ensure the production of two different daughter ions, the molecular ions have to contain at least one ^{37}Cl. The analytical quality criterion for screening technique allows a broader range of isotopic ratios (i.e. ± 25%). In addition, it should be added that the labelled product ions are characterized by [M+11], as one carbon is lost during fragmentation.

Table 3-1 : Main parameters optimized for MS/MS analysis of dioxins and furans. The congener's classification corresponds to the elution order on Rtx5-MS 40 m column.

Peak	Compounds	Window (min)	Molecular ions	CID (V)	Collision time (ms)	q value	Product ions	Isotopic ratios
1	**2,3,7,8 TCDF**	20-21.4	306 [M+2]	5.5	30	0.45	241/243	0.33
	2,3,7,8 TCDF $^{13}C_{12}$		318 [M+2]	5.5	30	0.45	252/254	0.33
2	**2,3,7,8 TCDD**	21.4-21.95	322 [M+2]	5	30	0.45	257/259	0.33
	2,3,7,8 TCDD $^{13}C_{12}$		334 [M+2]	5	30	0.45	268/270	0.33
3	**1,2,3,7,8 PeCDF**	21.95-25.7	340 [M+2]	6	30	0.45	275/277	0.25
	1,2,3,7,8 PeCDF $^{13}C_{12}$		352 [M+2]	6	30	0.45	286/288	0.25
4	**2,3,4,7,8 PeCDF**		340 [M+2]	6	30	0.45	275/277	0.25
	2,3,4,7,8 PeCDF $^{13}C_{12}$		352 [M+2]	6	30	0.45	286/288	0.25
5	**1,2,3,7,8 PeCDD**	25.7-29	356 [M+2]	6	30	0.45	291/293	0.25
	1,2,3,7,8 PeCDD $^{13}C_{12}$		368 [M+2]	6	30	0.45	302/304	0.25
6	**1,2,3,4,7,8 HxCDF**	29-33.5	374 [M+2]	6	30	0.45	309/311	0.20
	1,2,3,4,7,8 HxCDF $^{13}C_{12}$		386 [M+2]	6	30	0.45	320/322	0.20
7	**1,2,3,6,7,8 HxCDF**		374 [M+2]	6	30	0.45	309/311	0.20
	1,2,3,6,7,8 HxCDF $^{13}C_{12}$		386 [M+2]	6	30	0.45	320/322	0.20
8	**2,3,4,6,7,8 HxCDF**		374 [M+2]	6	30	0.45	309/311	0.20
	2,3,4,6,7,8 HxCDF $^{13}C_{12}$		386 [M+2]	6	30	0.45	320/322	0.20
9	**1,2,3,4,7,8 HxCDD**		390 [M+2]	6	30	0.45	325/327	0.20
	1,2,3,4,7,8 HxCDD $^{13}C_{12}$		402 [M+2]	6	30	0.45	336/338	0.20
10	**1,2,3,6,7,8 HxCDD**		390 [M+2]	6	30	0.45	325/327	0.20
	1,2,3,6,7,8 HxCDD $^{13}C_{12}$		402 [M+2]	6	30	0.45	336/338	0.20
11	**1,2,3,7,8,9 HxCDD**		390 [M+2]	6	30	0.45	325/327	0.20
	1,2,3,7,8,9 HxCDD $^{113}C_{12}$		402 [M+2]	6	30	0.45	336/338	0.20
12	**1,2,3,7,8,9 HxCDF**		374 [M+2]	6	30	0.45	309/311	0.20
	1,2,3,7,8,9 HxCDF $^{13}C_{12}$		386 [M+2]	6	30	0.45	320/322	0.20
13	**1,2,3,4,6,7,8 HpCDF**	33.5-37.5	410 [M+4]	6	30	0.45	345/347	0.40
	1,2,3,4,6,7,8 HpCDF $^{13}C_{12}$		422 [M+4]	6	30	0.45	356/358	0.40
14	**1,2,3,4,6,7,8 HpCDD**		426 [M+4]	6	30	0.45	361/363	0.40
	1,2,3,4,6,7,8 HpCDD $^{13}C_{12}$		438 [M+4]	6	30	0.45	372/374	0.40
15	**1,2,3,4,7,8,9 HpCDF**		410 [M+4]	6	30	0.45	345/347	0.40
	1,2,3,4,7,8,9 HpCDF $^{13}C_{12}$		422 [M+4]	6	30	0.45	356/358	0.40
16	**OCDD**	37.5-43	460 [M+4]	6	30	0.45	395/397	0.33
	OCDD $^{13}C_{12}$		472 [M+4]	6	30	0.45	406/408	0.33
17	**OCDF**		444 [M+4]	6	30	0.45	379/381	0.33
	OCDF $^{13}C_{12}$		456 [M+4]	6	30	0.45	390/392	0.33

During multiple reaction monitoring mode, the instrument alternatively scans the native and labelled region within the time window and ion current can be reconstructed from those channels.

Figure 3-4 shows the chromatogram for 2,3,7,8 TCDF. The top trace shows the total ion current (TIC) for the native TCDF while the trace below shows the TIC for $^{13}C_{12}$ internal standard. They coelute and the Figure 3-4 illustrates the alternation of MS/MS events. The ion current can be reconstructed by their corresponding product ions for quantification (i.e. 241+243 and 252+254) as shown on bottom traces.

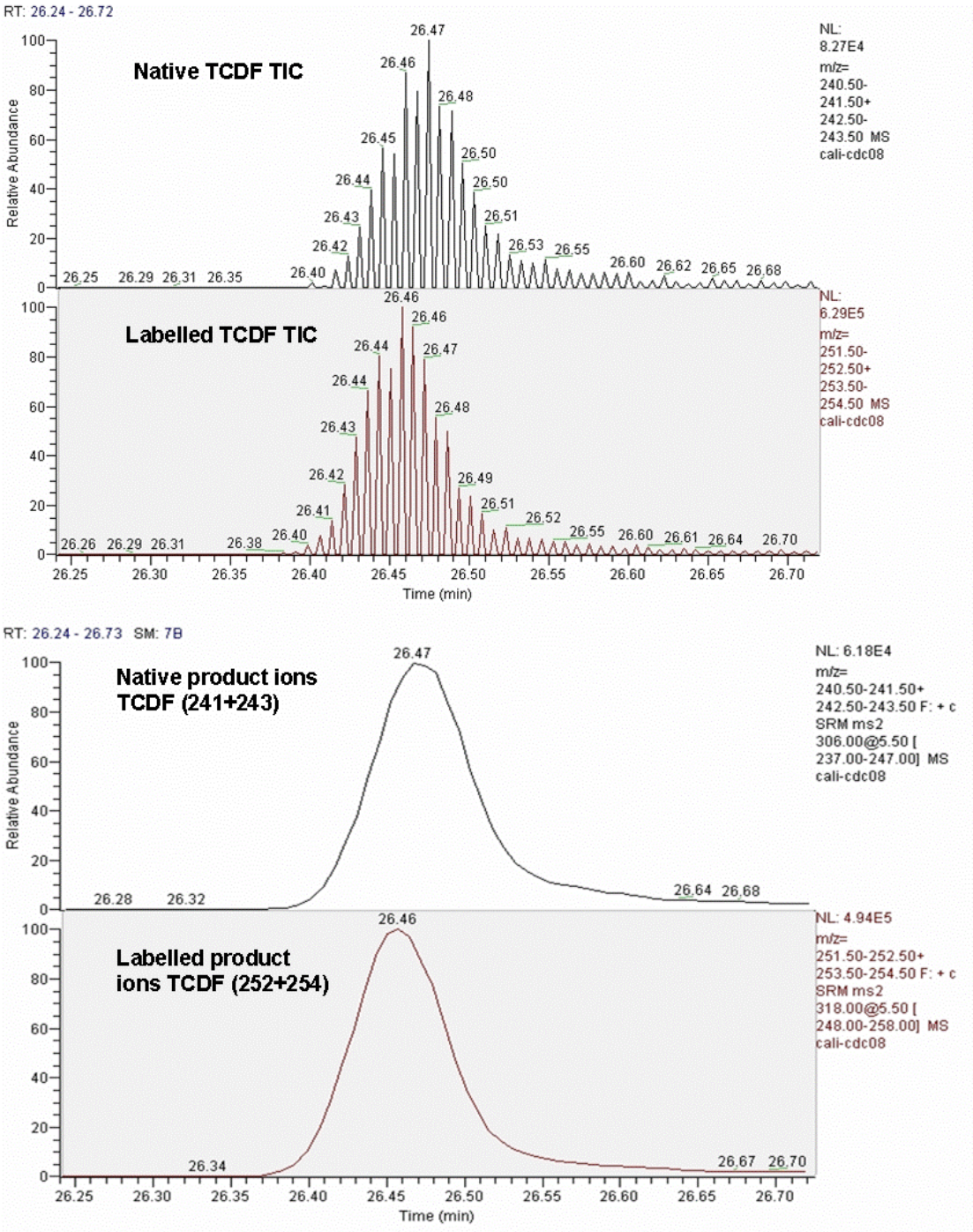

Figure 3-4 : MRM mode for native TCDF and its labelled $^{13}C_{12}$ internal standard

Calibration is carried out by injecting a five-points calibration solution by PTV-LV-GC/MS/MS. The linear response range (i.e. in the working range) as well as the relative response factor (RRF) associated to each congener is established. The RRF value is calculated here as follow:

$$RRF = \frac{(A^1_{native,i} + A^2_{native,i}) \times C_s}{(A^1_{std,i} + A^2_{std,i}) \times C_n} \quad (3\text{-}1)$$

Where A^1_{native} and A^2_{native} are the areas of the primary and secondary product ion of the native congener ; A^1_{std} and A^2_{std} are the areas of the primary and secondary product ion of the internal ^{13}C standard; C_n is the concentration of the native compound in the calibration solution and C_s is the concentration of the labelled compound in the calibration solution.

When the concentration ratio of native to analogue standard ^{13}C is plotted against the area ratio of native to analogue standard ^{13}C, the slope of the curve gives the corresponding RRF value. Good linearity is achieved and regression lines were characterised by correlation coefficients (R^2) higher than 0.99 for all the congeners. Table 3-2 gives an overview of the average RRFs and their corresponding coefficients of variation (CVs) calculated during a calibration of the 17 PCDD/F congeners. The values are compared to the results obtained during a classical GC/HRMS calibration exercise. As it can be seen, CVs in the range of 2.3% and 21% were obtained by the alternative method. CVs are generally higher than those obtained by the reference HRMS method but are still acceptable.

Table 3-2 : Average RRFs and their corresponding CVs for individual congener calibrations by PTV-LV-GC/MS/MS and GC/HRMS.

	MS/MS	CV	HRMS	CV
Compounds	**RRF**	**%**	**RRF**	**%**
2,3,7,8 TCDD	1.01	2.3	1.02	7.0
1,2,3,7,8 PeCDD	0.86	10.9	0.92	9.7
1,2,3,4,7,8 HxCDD	1.08	9.2	1.09	6.1
1,2,3,6,7,8 HxCDD	0.79	15.6	0.88	4.8
1,2,3,7,8,9 HxCDD	0.92	8.5	1.00	5.2
1,2,3,4,6,7,8 HpCDD	1.09	7.0	0.91	6.0
OCDD	0.99	3.0	1.04	3.1
2,3,7,8 TCDF	0.87	9.2	0.94	2.5
1,2,3,7,8 PeCDF	0.84	4.3	0.84	6.8
2,3,4,7,8 PeCDF	0.82	13.7	0.98	3.8
1,2,3,4,7,8 HxCDF	1.11	5.8	1.03	5.7
1,2,3,6,7,8 HxCDF	1.08	11.4	0.94	3.4
1,2,3,7,8,9 HxCDF	0.98	7.0	0.94	7.3
2,3,4,6,7,8 HxCDF	1.00	15.4	1.02	6.7
1,2,3,4,6,7,8 HpCDF	1.02	4.7	1.05	4.1
1,2,3,4,7,8,9 HpCDF	1.10	16.5	0.75	10.0
OCDF	1.09	21.0	1.07	8.2

The individual 2,3,7,8 PCDD/F congener quantification is then calculated as follow:

$$[congener]_i = \frac{(A^1_{native,i} + A^2_{native,i}) \times Q_i}{(A^1_{std,i} + A^2_{std,i}) \times RRF_i \times m} \quad (3\text{-}2)$$

Where $[congener]_i$ is the concentration of the congener i (ng/kg); Areas are defined above (equation 3-1) ; Q_i is the amount of the corresponding internal standard i spiked (ng) in the sample ; RRF_i is the relative response factor of the congener i and m is the weight of the sample (kg). Finally, the quantification in TEQ is calculated using the toxic equivalence factors (TEFs) reported by the World Health Organisation (1998).

3.3.1 Improvement of the alternative method sensitivity

The first applications of the method started with environmental matrices which contain higher levels of dioxin compared to food and feed. In 1990, Reiner had reported the complementary of MS/MS (triple quadrupole and ion trap) and HRMS techniques for the analysis of ultra trace levels of PCDD/Fs in environmental matrices (Reiner et al., 1990). Both methods can filter out different interferences; however, neither technique can remove all interferences. March and co-workers were the first to report identification and quantification of 2,3,7,8 TCDD (Plomley et al., 1994). Afterwards, we also showed for both GC-MS/MS and GC/HRMS the complementarity of the techniques for dioxin analysis in fly ashes (Eppe and De Pauw, 1997, Focant et al., 2001). This alternative method can match the required sensitivity, selectivity and specificity for environmental matrices. In 1994, Plomley had already reported a value of 500 fg injected (with a signal to noise ratio of 5:1) for the detection of 2,3,7,8 TCDD by ion trap MS/MS technique (Plomley et al., 1994). However, the instrument detection limit (IDL) cannot be compared with HRMS instrument. Indeed, a typical specification supplied with a Micromass Autospec Ultima high resoltution mass spectrometer is 100 fg with a S/N ratio 100:1 in SIM mode for 321,8937 Da (TCDD). In routine conditions (with longer column and more ions selected in the time window), our sensitivity criteria for routine HRMS injection is 80 fg with a S/N ratio of 10:1. In comparison, in a recent report on sensitivity specification for 2,3,7,8 TCDD using a quadrupole ion trap in MS/MS mode (Hayward, 2002), the author examined the optimisation of 26 parameters with the aim of setting a benchmark sensitivity for a selected ion trap instrument. An average signal to noise ratio of 49:1 for 400 fg of 2,3,7,8 TCDD injected was achieved in optimal conditions (i.e. not routine conditions).

All the sensitivity tests performed and reported in the literature for TCDD using either an internal or external ionisation system for ion trap generally agree with the criteria that the IDL is approximately 5 to 10 times higher than the IDL obtained with an HRMS sector instrument.
The aim of the present study was to build up an alternative method able to provide reliable results in food and feed matrices. Two ways were investigated to partially fill in the gap of sensitivity between both techniques. First, sensitivity improvements on the ion trap itself were studied by increasing the trapping efficiency of ions. The second approach considered was to inject higher amount into the GC column by coupling GC with large volume injection.
One should note that a third possible approach consisting of increasing the sample intakes was not further investigated because this option didn't match the requirements of a screening approach based on high throughput capacities.

3.3.1.1 Damping gas pressure inside the trap

Recent developments in trapping efficiency inside the ion trap mass spectrometer permitted to lower the IDL. Sensitivity improvement was obtained by an optimisation of the damping gas pressure of helium inside the trap. During this thesis, we developed in collaboration with Thermo the first prototype system on a PolarisQ that allows a control regulation flow of Helium inside the trap. Here, ions are formed in an external ion source by electron ionisation (EI) mode. They are then accelerated through three lenses and introduced into the ion trap analyser. The RF voltage applied to the ring electrode is set to a value that is optimised for optimum trapping efficiency. A buffer gas of helium is continually introduced into the ion trap to slow the motion of ions by collision for better trapping efficiency, a process called relaxation. Figure 3-5 shows that the kinetic energy allowing the ions to enter the ion trap allows them to subsequently escape. To remove kinetic energy from the ions, approximately 1 mTorr of helium buffer gas must be present in the ion trap. Collisions remove kinetic energy and allow ions to be trapped. The sensitivity is therefore limited here by the trapping efficiency of the precursor ion. To improve the trapping efficiency of the precursor ion, a new hardware that increases the helium damping gas pressure inside the ion trap was installed and evaluated on the instrument. The helium damping gas flow rate inside the trap was preset to a default value of 0.3mL/min. By increasing the helium pressure to a flow rate of approximately 1.7mL/min (i.e. the optimum), the trapping efficiency of the precursor ion was enhanced; hence increasing the product ion yield in MS/MS mode.

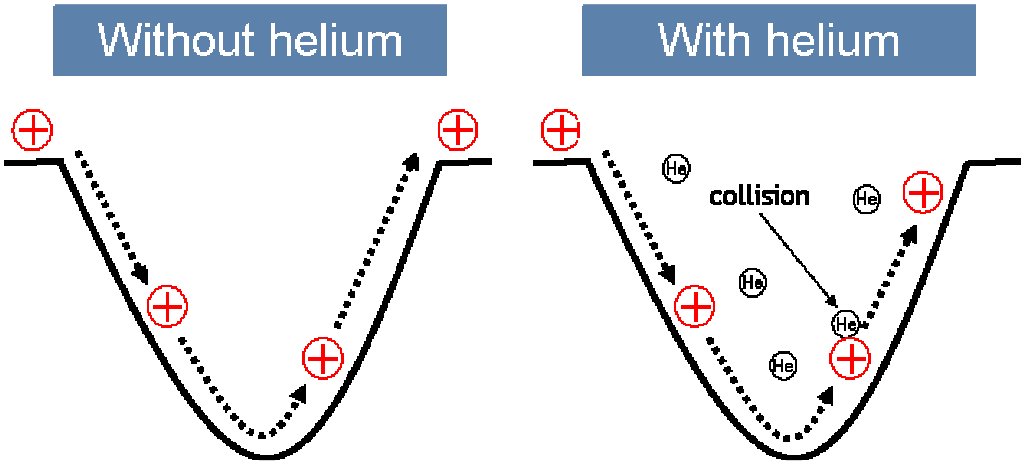

Figure 3-5 : Representation of ions trapped in a potential well for quadrupole ion trap MS (Thermo)

Before the isolation step was complete, an optimum of the flow rate was needed in order to prevent precursor ion from fragmentation. The second role of Helium was to operate as a collision gas during the collision-induced dissociation (CID) process, which causes the fragmentation of precursor ions in product ions. Due to the high helium damping gas pressure inside the trap, precursor ion motion is damped and therefore it takes more energy to excite the ions for CID fragmentation. Voltages between 5 and 6 Volts were applied to the endcap electrodes to fragment tetra-through octa-chlorinated dioxins and furans (as reported in Table 3-1). The product ions masses, characterized by a loss of $COCl^{\cdot}$, are then scanned by ramping a resonance ejection RF voltage that sequentially eject them from low to high m/z.

Thus, the IDL defined as signal to noise ratio was improved by a factor 2 to 3 for 2,3,7,8-TCDD in MS/MS mode compared to the value already reported (Plomley et al., 1994). Figure 3-6 shows the chromatogram for 200 fg of 2,3,7,8-TCDD injected in routine conditions. The chromatogram was rebuilt with the merged ion current of the two product ions (257+259 m/z). Compared to HRMS in routine conditions, 80 fg with S/N >10 was achieved. Thus, the option of injecting a higher amount of sample into the GC column by PTV injection was attractive. We investigated this option, keeping in mind that the more injected, the more risk taken to contaminate the column and the ion trap. A specific and dedicated clean-up is therefore needed for large volume injection.

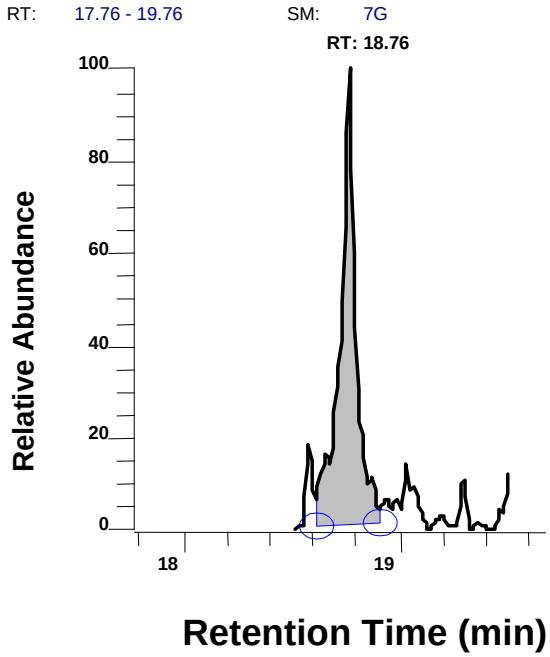

Figure 3-6 : Instrument Detection Limit of 200 fg 2,3,7,8-TCDD (m/z : 257+259) with S/N ratio ≥ 5:1 by PTV-LV-GC/MS/MS.

3.3.1.2 PTV-LV injection for PCDD/Fs analysis.

Basically, a programmed temperature vaporizer injector (PTV) is a split-splitless injector with temperature control, i.e., the vaporizer chamber can be heated or cooled rapidly. Three types of large-volume introduction techniques can be distinguished: PTV solvent split injection, PTV large-volume splitless injection and PTV vapour overflow. The first mode is the most commonly used technique. It consists in the injection of the sample in a packed liner with an open split exit at an injector temperature just below the solvent boiling point. Volatile compounds co-evaporating with the solvent are lost. After solvent evaporation, the analytes retained in the liner are transferred to the GC column in splitless mode.

Large volume injection into a PTV injector can be done in different modes: ‘at once’ or by a ‘speed control injection’ and even using ‘multiple injection’. The first two modes of injection are normally preferred with the type of PTV used here. ‘At once’, the sample is introduced at a relatively high speed (e.g. 10µL/sec) and the maximum introduction volume that can be injected mainly depends on the liner dimensions: a 1 mm i.d. liner can hold 20-30µL of liquid. The speed control injection mode introduces the sample at a rate that is theoretically equal to the evaporation rate. Higher sample volumes can be introduced by this manner. Both modes

have been tested and best results were obtained by injecting the sample at a speed control of 2.1µL/sec.

Toluene was used to optimise the PTV parameters. This solvent was selected because PCDD/Fs are collected from the last clean-up step in toluene (see paragraph 2.3.3). It is characterized by a high boiling point (i.e. 110°C) and therefore is not the easiest solvent to use for large volume injection. PTV injection is divided into 4 phases: the injection, the vaporization, the transfer and finally the cleaning phase. During the injection, the split valve is open and the sample is introduced into the cold liner filled with glass wool set at a temperature below the boiling point of toluene (100°C). PCDD/Fs are characterized by high boiling points and no significant losses occurred at 100°C. During the evaporation step, the PTV temperature is raised to 120°C for 30 sec in order to eliminate the solvent. The solvent is vented through the split valve at an optimized split flow of 100mL/min. Once the solvent is eliminated, the third step consists in transferring the components to the analytical column. The split valve is closed and the temperature is rapidly raised to 300°C in splitless mode for 1 min. After transfer of the components, the split valve is opened again (100mL/min) and the liner is kept at 300°C during the GC run for cleaning. Optimized parameters are summarized in Table 3-3.

The first trials were performed with 30µL of toluene. Toluene is difficult to pump out; it remains in the GC/MS system for a minimum of 2 hours after injection. An alternative option to solve the problem was to equip the system with a back-flush device for PTV injection. If considerable improvements for toluene were noticed, unfortunately significant losses of components were also observed. The reason is mostly due to the use of a high boiling point solvent. Back-flush applications for PTV injection seem to be suitable with the lower boiling point solvent (e.g. pentane, hexane).

Table 3-3 : PTV-LV injection parameters optimized for PCDD/Fs analysis

PTV-LV 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	40 min
Cleaning flow	100 mL/min

The problem was finally partially solved by evaporating the final extract to a lower volume and injecting 10µL into the PTV injector.

3.3.2 Comparison between the PTV-LV-GC/MS/MS method and the reference method

This paragraph deals with the comparison between the optimised alternative method and the reference method. The approach proposed here consisted in comparing the performances of both methods on five different food and feed matrices in a TEQ working range from background to contaminated levels. The second step of the validation consisted in an external validation for PCDD/Fs and DL PCBs in food and feed based on the participation in an inter-comparison exercise with other expert laboratories in the framework of a European project (DIFFERENCE). We participated in this study with four different analytical techniques: GC/HRMS, PTV-LV-GC/MS/MS, GCXGC-TOFMS and bioassay CALUX approach. For this thesis, we focused on the comparison of the alternative PTV-LV-GC/MS/MS method with GC/HRMS.

3.3.2.1 Comparison between PTV-LV-GC/MS/MS and GC/HRMS on different matrices by ANOVA

The experimental section describing in details the sample preparation and the analysis by both techniques is attached (see related paper).

i. Quality control samples and certified reference material

The evaluation of the screening method is based on the simultaneous comparison of means' values obtained for both techniques on different types of samples and levels. Five different matrices have been selected: beef fat, yolk, foetal bovine serum, animal feed, and milk powder. All the samples are quality control (QC). Beef fat, eggs yolk, and serum samples were fortified with PCDD/Fs at different levels whereas the animal feed QC was naturally contaminated with dioxins and furans. The last selected matrix was a certified reference material BCR-607 (IRMM, Geel, Belgium), a spray-dried milk powder. The dioxin levels in TEQ for these five matrices spanned two orders of magnitude (i.e. from 0.2 to 25 pg-TEQ/g). For each matrix, a series of ten replicates was carried out. Five samples out of the ten were analysed by the PTV-LV-GC/MS/MS method while the remaining five samples were injected into the GC/HRMS instrument. Thus, a total of 50 analyses have been performed.

ii. Statistical evaluation

The validation consisted of comparing the PTV-LV-GC/MS/MS alternative method to the GC/HRMS method on five different types of samples (i.e. beef fat, yolk, milk powder, animal feed and serum). Matrices were chosen to cover commonly encountered interferences. The sample intakes and the sample preparation were similar for both analytical techniques. Furthermore, five replicates per method were carried out. An overview of the mean values with their corresponding standard deviations (SDs) is presented in Table 3-4. For each individual congener, results were expressed in parts-per-trillion (ng/kg) but they are also presented in WHO-TEQ for the sum of the 17 congeners. As it can be seen, the PCDD/Fs levels in TEQ vary from 0.2 to 25 ngWHO-TEQ/kg. Good agreement between the mean values in TEQ was found, even if higher standard deviations for the screening method were obtained. This remark was also true for individual congeners. If Relative Standard Deviations (RSDs under repeatability conditions) are calculated, they vary in TEQ from 6.2 to 17.1% for the different matrices. For individual congeners, most of the RSDs are in the range between 4 to 40% except some of them that can increase to more than 100%.

At this stage, if we conduct a comparison between means per congener and also per matrix, 74 tests of means comparison should be necessary (see Table 3-4). In order to be able to draw global conclusions, statistical treatment of data using an analysis of variance allows a simultaneous comparison of means (Feinberg, 1996). For multi-factor experimental designs, ANOVA can provide separate variance estimates for each factor.

The general equation used here for the ANOVA is the following:

$$SCE_t = SCE_r + SCE_a + SCE_b + SCE_{ab} \quad (3\text{-}3)$$

Where SCE_t is the sum of the square of the total deviations ; SCE_r is the sum of the square of the residual deviations; SCE_a is the sum of the square of the deviations due to the method factor ; SCE_b is the sum of the square of the deviations due to the matrix factor, SCE_{ab} is the sum of the square of the deviations due to the interaction between the method and the matrix. The ANOVA can also take into account possible interactions effects (e.g. between method and matrix).

In our example, several types of means were calculated: means per sample, means per matrix or means per method. Thus, to test the comparison between means, hypotheses tests are interesting statistical tools to see if our claims are correct. These tests were developed to facilitate the decision-making at a significant level. The first step was to specify the null hypothesis (H_0). The purpose of this study was to assume the equality between means:

$H_0 : \mu_1 = \mu_2 = \ldots = \mu_n$ (3-4)

The alternative hypothesis (H_1) would be:

$H_1 : \mu_1 \neq \mu_2 \neq \ldots \neq \mu_n$ (3-5)

The second step was to select a significance level for rejection of H_0. A typical level of α = 0.05 was chosen. As mentioned above, the ANOVA can be used to test the comparison between multiple means. In addition, it can provide separate variance estimates for each parameter. The comparison between two variances was done by the statistical test of Fisher. In the third step, the statistic test value (i.e. the calculated F value) was then used to decide whether or not the null hypothesis should be rejected in our hypothesis test at the significance level. This was done by comparing the calculated F value to its critical tabulated F value. In our validation study, three different F values were calculated per congener: F_{method}, F_{matrix} and $F_{interaction}$. Fisher test values were calculated by dividing the parameter variance (i.e. method, matrix or interaction) by the residual variance.

In the statistical hypothesis test, the probability value (p-value) is often used. Small p-values suggest that the null hypothesis is unlikely to be true. If p-values are smaller than the pre-established significance level, then the null hypothesis is rejected, suggesting that the alternative hypothesis may be true.

ANOVA results are presented in Table 3-5. For each congener, the calculated F values, their corresponding critical tabulated F values and the p-values are reported. The interpretation of the results indicates that F_{method} is always below its critical F value. The method effect is therefore not significant for the 17 PCDD/Fs. The null hypothesis is not rejected and this is confirmed by high probability p-values. Nevertheless, three congeners (2,3,7,8-TCDF; 1,2,3,4,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD) have p-values below 0.5 indicating that there is only less than 0.5 chance that H_0 is true.

On the other hand, the matrix effect is significant for all the congeners ; H_0 is rejected. As dioxin and furan levels in the different matrices were sometimes covering several orders of magnitude, the rejection of H_0 is obvious. Besides, the interaction between method and matrix pointed out that these effects are pure (interaction is not significant) for most of the 2,3,7,8 congeners. H_0 is not rejected and it brings out the good selectivity of the alternative method for most of them. However, the 3 congeners, mentioned above, have p-values smaller than the significance level ($p<0.05$). It indicates the influence of specific matrix on the mean values for the comparison between both techniques. For instance, 2,3,7,8-TCDF mean value in beef fat is greatly higher by PTV-LV-GC/MS/MS analysis than by GC/HRMS analysis (Table 3-4). It might be coming from specific matrix interference.

Table 3-4 : PCDD/Fs mean values expressed in parts-per-trillion (ng/kg) and their corresponding standard deviations for PTV-LV-GC/MS/MS and GC/HRMS methods

	Beef Fat				Yolk				Milk powder BCR 607					Animal Feed				Bovine Serum			
	MS/MS		HRMS		MS/MS		HRMS		MS/MS		HRMS			MS/MS		HRMS		MS/MS		HRMS	
	ng / kg	SD	ng / kg	SD	ng / kg	SD	ng / kg	SD	ng / kg	SD	ng / kg	SD	Assigned Value	ng / kg	SD	ng / kg	SD	ng / kg	SD	ng / Kg	SD
Dioxins/Furans	mean	n=5	mean	n=5	mean	n=5	mean	n=5	mean	n=5	mean	n=5	ng/kg	mean	n=5	mean	n=5	mean	n=5	mean	n=5
2,3,7,8 TCDD	0.28	0.06	0.38	0.04	2.07	0.42	2.37	0.18	0.24	0.03	0.29	0.01	0.25	nd		nd		0.02	0.00	0.02	0.00
1,2,3,7,8 PeCDD	2.04	0.32	1.71	0.11	9.79	1.87	8.68	0.35	0.78	0.12	0.81	0.06	0.79	0.08	0.06	0.02	0.02	0.10	0.02	0.08	0.01
1,2,3,4,7,8 HxCDD	2.04	0.14	1.82	0.17	7.63	0.54	9.36	0.39	0.45	0.18	0.46	0.04	0.42	0.29	0.19	0.14	0.04	0.09	0.01	0.07	0.02
1,2,3,6,7,8 HxCDD	2.07	0.27	2.25	0.11	10.65	2.55	10.64	0.09	1.02	0.07	1.09	0.09	0.98	2.38	0.44	2.27	0.14	0.11	0.02	0.10	0.01
1,2,3,7,8,9 HxCDD	1.81	0.21	1.95	0.04	7.96	1.41	9.56	0.31	0.30	0.25	0.38	0.03	0.34	0.76	0.16	0.84	0.12	0.12	0.03	0.08	0.01
1,2,3,4,6,7,8 HpCDD	2.02	0.31	1.50	0.19	9.18	1.05	6.19	0.09	nd		nd		not assig.	144.2	6.99	166.4	12.18	0.09	0.02	0.11	0.02
OCDD	4.60	1.66	4.42	1.35	24.61	1.07	21.76	1.56	nd		nd		not assig.	962.7	158.1	927.7	124.1	0.19	0.05	0.21	0.01
2,3,7,8 TCDF	2.50	0.82	0.34	0.15	2.95	0.52	2.91	0.11	0.08	0.03	nd		0.05	0.11	0.06	0.11	0.03	0.09	0.03	0.03	0.00
1,2,3,7,8 PeCDF	1.99	0.20	2.23	0.06	11.36	0.77	12.27	0.03	0.08	0.03	nd		0.05	0.04	0.04	0.02	0.01	0.12	0.01	0.12	0.01
2,3,4,7,8 PeCDF	1.69	0.71	2.12	0.04	11.52	0.96	12.38	0.26	1.81	0.20	1.81	0.09	1.81	0.09	0.10	0.06	0.02	0.11	0.01	0.11	0.01
1,2,3,4,7,8 HxCDF	1.94	0.40	1.91	0.11	9.40	2.08	9.63	0.06	0.84	0.08	0.92	0.04	0.94	0.14	0.10	0.08	0.01	0.10	0.02	0.09	0.00
1,2,3,6,7,8 HxCDF	2.14	0.17	1.95	0.09	9.75	1.61	10.89	0.48	1.03	0.15	1.08	0.04	1.01	0.04	0.04	0.05	0.01	0.04	0.01	0.10	0.01
1,2,3,7,8,9 HxCDF	2.28	0.58	2.21	0.17	10.55	1.26	11.14	0.27	nd		nd		not assig.	nd		nd		0.10	0.02	0.10	0.01
2,3,4,6,7,8 HxCDF	1.97	0.37	1.93	0.12	10.63	1.37	10.69	0.35	1.03	0.12	1.07	0.08	1.07	nd		0.04	0.02	0.08	0.01	0.10	0.01
1,2,3,4,6,7,8 HpCDF	1.89	0.52	2.14	0.09	8.91	2.30	11.34	0.57	nd		nd		not assig.	1.93	0.32	1.70	0.28	0.13	0.01	0.09	0.00
1,2,3,4,7,8,9 HpCDF	1.65	0.28	2.14	0.13	9.02	0.79	10.21	0.43	nd		nd		not assig.	0.24	0.23	0.21	0.06	0.08	0.02	0.09	0.01
OCDF	5.14	0.87	3.57	0.23	18.65	1.57	17.85	0.30	nd		nd		not assig.	11.46	0.78	13.55	1.54	0.13	0.03	0.15	0.03
WHO-TEQ-PCDD/F	**5.10**	0.32	**4.72**	0.22	**25.42**	2.36	**25.61**	0.23	**2.24**	0.38	**2.50**	0.08	**2.43**	**2.15**	0.13	**2.20**	0.23	**0.26**	0.03	**0.23**	0.02

Table 3-5 : Synthesis of ANOVA results using Fisher test for the 17 PCDD/F congeners

Dioxins/Furans	Calculated F values			Tabulated F values at the 95% significance level			p-values (α =0.05)		
	Method	matrix	interaction	Method	matrix	interaction	Method	matrix	interaction
2,3,7,8 TCDD	0.2346	38.77	0.313	4.17	2.92	2.92	0.6314	<0.05	0.8158
1,2,3,7,8 PeCDD	0.0918	30.98	0.228	4.08	2.61	2.61	0.7640	<0.05	0.9205
1,2,3,4,7,8 HxCDD	0.6845	232.09	6.117	4.08	2.61	2.61	0.4138	<0.05	<0.05
1,2,3,6,7,8 HxCDD	0.0001	23.39	0.007	4.08	2.61	2.61	0.9921	<0.05	0.9999
1,2,3,7,8,9 HxCDD	0.2412	53.93	1.015	4.08	2.61	2.61	0.6263	<0.05	0.4126
1,2,3,4,6,7,8 HpCDD	0.6011	316.16	3.779	4.17	2.92	2.92	0.4442	<0.05	<0.05
OCDD	0.0085	43.39	0.029	4.17	2.92	2.92	0.9271	<0.05	0.9932
2,3,7,8 TCDF	1.3020	15.04	4.638	4.17	2.92	2.92	0.2623	<0.05	<0.05
1,2,3,7,8 PeCDF	0.4106	336.58	1.018	4.17	2.92	2.92	0.5269	<0.05	0.3995
2,3,4,7,8 PeCDF	0.0029	5.85	0.018	4.08	2.61	2.61	0.9574	<0.05	0.9993
1,2,3,4,7,8 HxCDF	0.0004	7.57	0.003	4.08	2.61	2.61	0.9841	<0.05	0.9999
1,2,3,6,7,8 HxCDF	0.0102	8.59	0.063	4.08	2.61	2.61	0.9201	<0.05	0.9924
1,2,3,7,8,9 HxCDF	0.0029	6.56	0.013	4.35	3.49	3.49	0.9575	<0.05	0.9871
2,3,4,6,7,8 HxCDF	0.0001	7.15	0.000	4.17	2.92	2.92	0.9921	<0.05	0.9999
1,2,3,4,6,7,8 HpCDF	0.0566	6.11	0.231	4.17	2.92	2.92	0.8136	<0.05	0.8741
1,2,3,4,7,8,9 HpCDF	0.0354	7.29	0.059	4.17	2.92	2.92	0.8520	<0.05	0.9808
OCDF	0.0002	5.34	0.102	4.17	2.92	2.92	0.9888	<0.05	0.9582
PCDD/Fs WHO-TEQ	8 E-06	6.51	0.0014	4.08	2.61	2.61	1.0000	<0.05	1.0000

Another possibility to interpret the raw data in the comparison between the two methods is the research of a functional relationship between the results (Feinberg, 1996). Establishing a linear relationship between the alternative method compared to the reference method enables the detection of bias. If the functional relationship is not merged with the bisecting line, two types of bias can be detected: a systematic bias or a bias per rotation. The first case is characterized by a constant bias between the methods; the second is proportional to the concentration level. Figure 3-7 shows the functional relationship between PTV-LV-GC/MS/MS method and GC-HRMS method for most of the 2,3,7,8 congeners. Two regression curves were plotted: the bisecting line and the functional relationship between the methods calculated by the least rectangular regression method. For the congener detected in the 5 matrices by both techniques, 25 raw data points are also represented on the graphs by a triangular shape. The statistical conclusions drawn for the three congeners characterized by low interaction p-values between method and matrix are graphically highlighted on Figure 3-7. A systematic bias is observed for the 2,3,7,8-TCDF congener whereas a rotation bias with increasing levels is noticed for 1,2,3,4,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD. Regarding the others congeners, some are practically merged with the bisecting line while others are characterized by a non-significant bias .

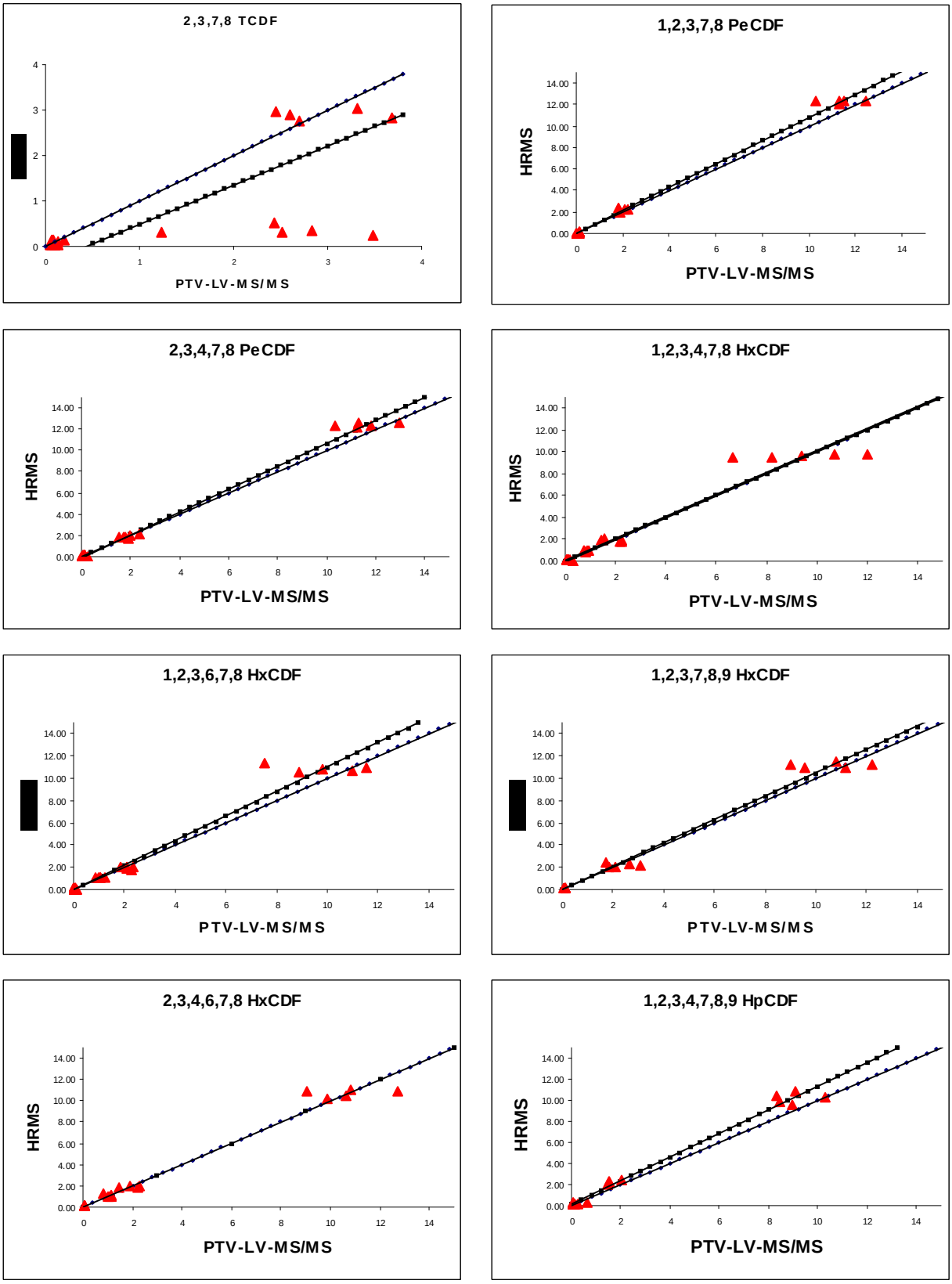

Figure 3-7 : Representation of the functional relationships between the PTV-LV-GC/MSMS method and the GC/HRMS method for PCDD/F congeners

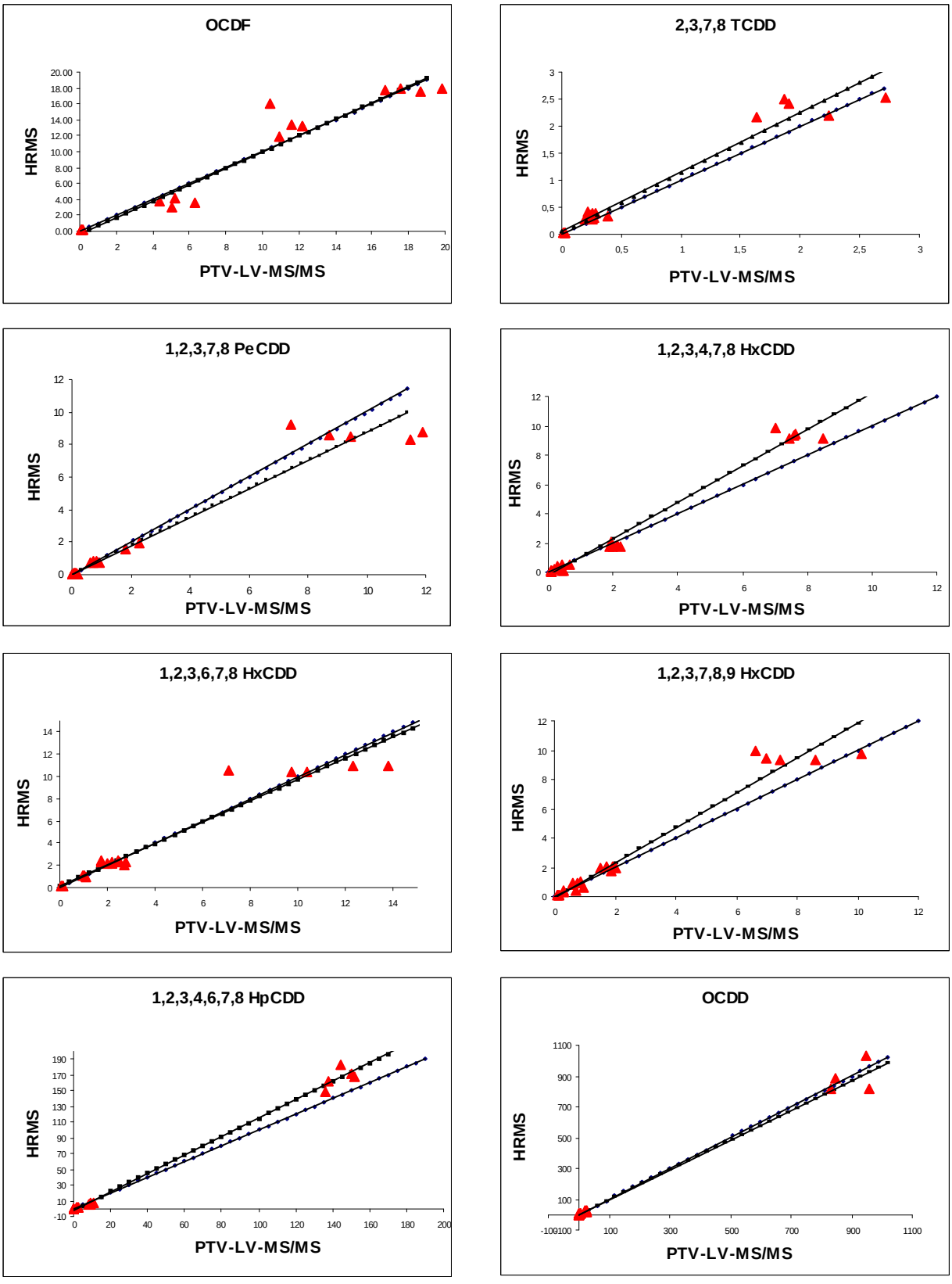

Figure 3-7 (continued)

In TEQ, the results indicate that, in the range of 0.2 to 25 ngWHO-TEQ/kg using five different matrices, no bias between the methods was observed as it can be seen in Figure 3-8. The functional relationship is merged with the bisecting line and the slope of the linear relationship is one.

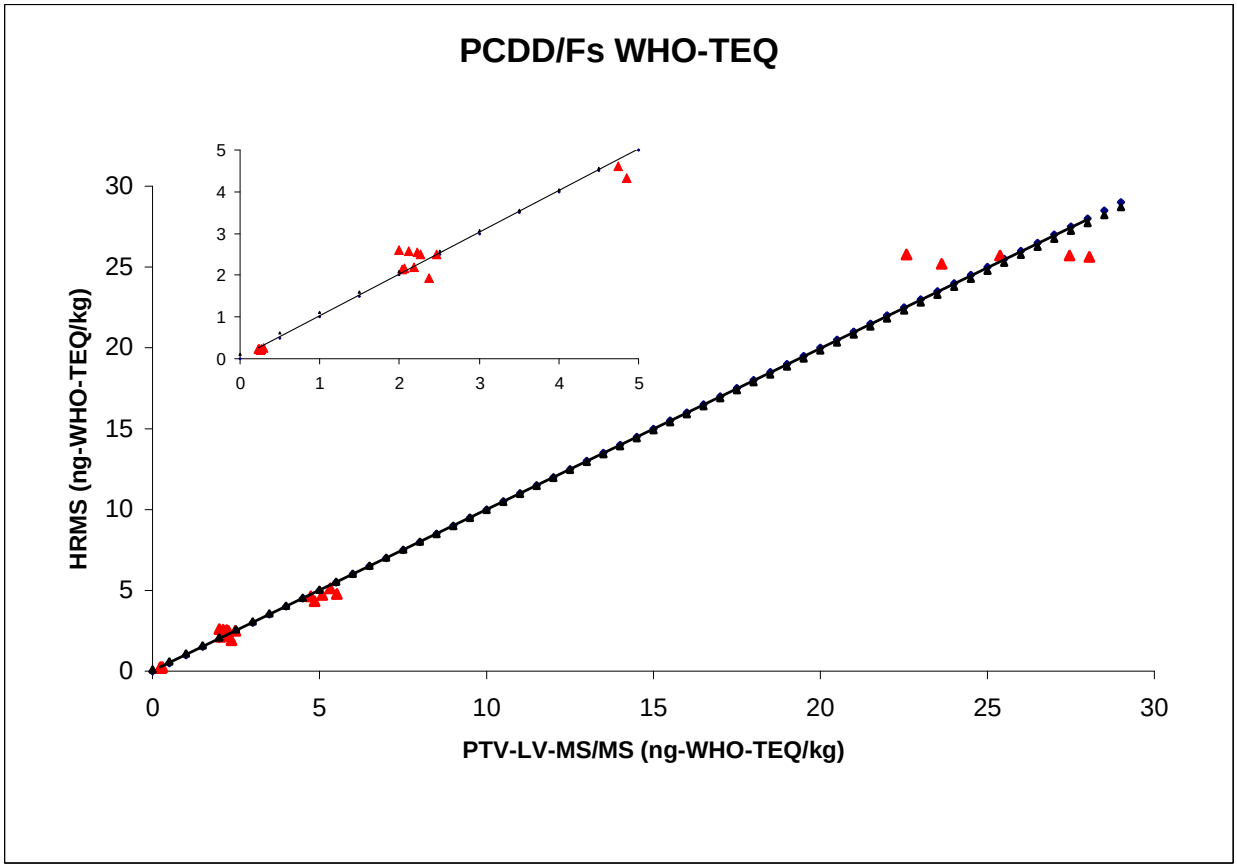

Figure 3-8 : Representation of the functional relationships between the PTV-LV-GC/MS/MS method and the GC/HRMS method in WHO-TEQ

3.3.2.2 Trueness assessment

The comparison between both techniques showed good agreement on TEQ basis in a range between 0.2 to 25 ng-WHO-TEQ/kg which is the working range of dioxin analysis in food and feed. The whole alternative method (samples intakes, extraction, clean-up, large volume injection and analysis) seems to be sensitive enough to detect low ppt levels even if the intrinsic sensitivity of the analyzer is lesser. However, the trueness of a method is determined usually by analyzing appropriate CRMs. Unfortunately, CRMs are scarce and expensive. But it is the best way to assess the trueness of an analytical method because there is a requirement for assigned values with a stated uncertainty, which are traceable to the same reference as the analytical results of the method used (Emons et al., 2004). CRMs provide exactly this traceable value. The CRM (BCR 607 milk powder) used in this study allows trueness

assessment according to these statements. The sample size for both methods was 10 g milk powder. Figure 3-9 shows a comparison between CRM assigned values and mean values obtained by PTV-LV-GC-MS/MS. Note that errors bars attached with mean values represent the repeatability standard deviation while the errors bars reported with assigned values represent the uncertainty at 95% confidence interval. All the mean values are within the trueness range, except for 1,2,3,4,7,8 HxCDF. In that case, a very small uncertainty is associated with the assigned value (0.94 ± 0.04) ng/kg but ranges are partially covered.

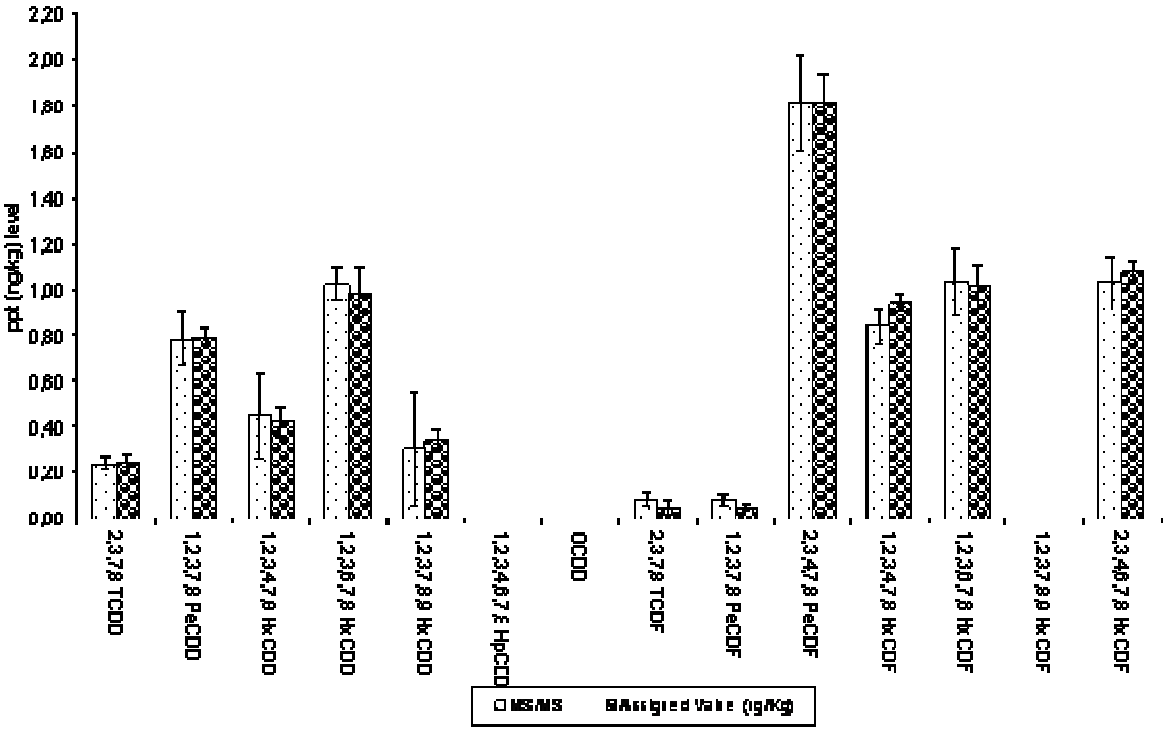

Figure 3-9 : Trueness assessment for some 2,3,7,8 PCDD/Fs congeners in BCR 607milk powder

Ideally, the trueness of the method should be assessed with appropriate CRMs covering the whole range and level of food matrices to consider this MS alternative method as suitable for dioxin analysis. During this thesis, we had the opportunity to participate at a feasibility study on five CRMs candidates in food and feed matrices (European project FP5, DIFFERENCE). The candidate materials were fish tissue, pork tissue, whole milk, fish oil and compound feed for pigs. Twelve European expert laboratories participated for assignment of certified values of dioxins, furans, DL-PCBs and indicator PCBs by HRMS method. Other laboratories participated with alternative techniques (GC/MS/MS, GCXGC-TOFMS, GCXGC-ECD, CALUX) to evaluate also the performances of potential screening techniques. We participated at the certification by HRMS but also with alternative techniques like PTV-LV-GC/MS/MS,

GCXGC-TOFMS and CALUX bioassay approach. The assessment of the trueness of the PTV-LV-MS/MS is presented here.

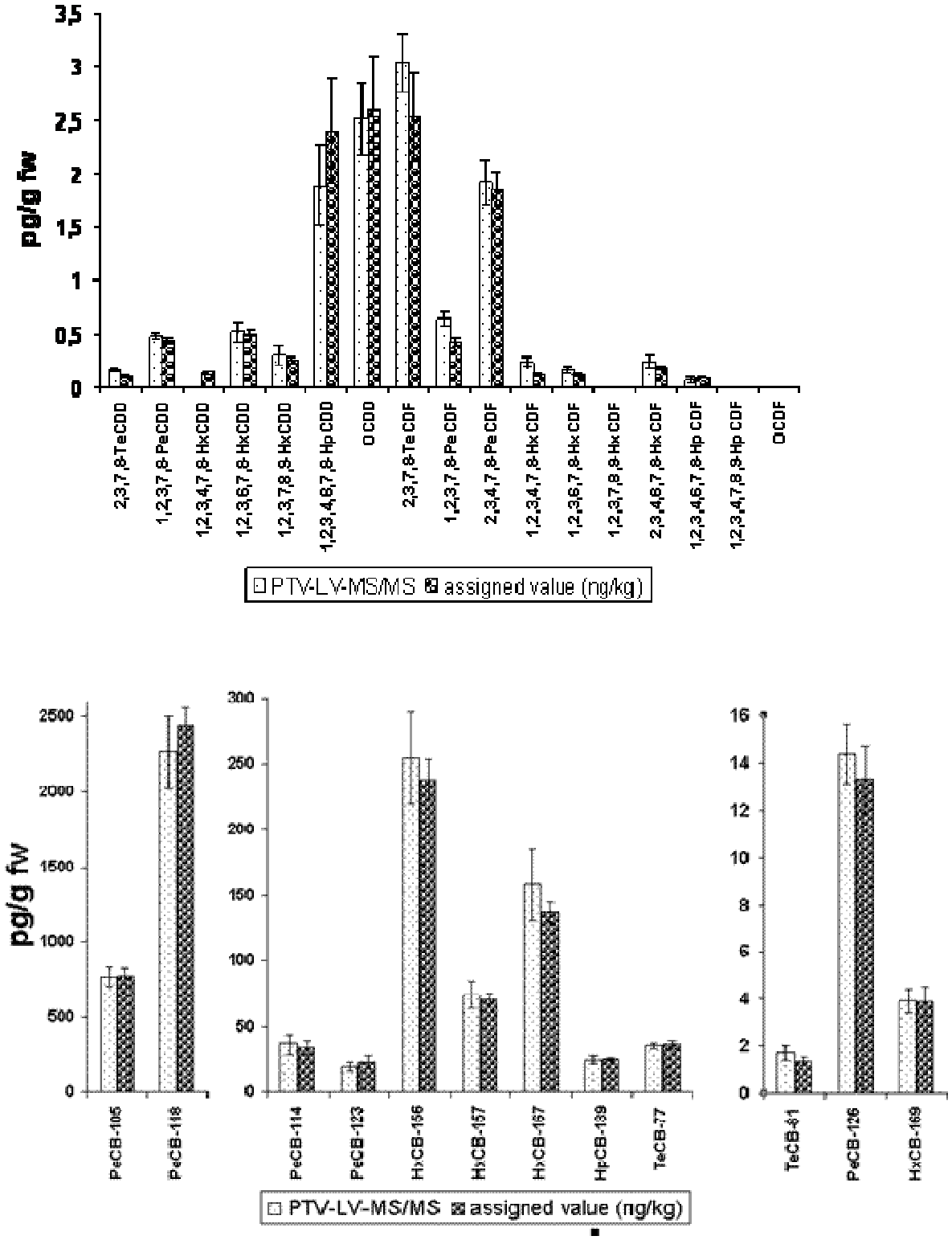

Figure 3-10 : Trueness assessment for PCDD/F and DL-PCB congeners in DIFF-01 fish tissue

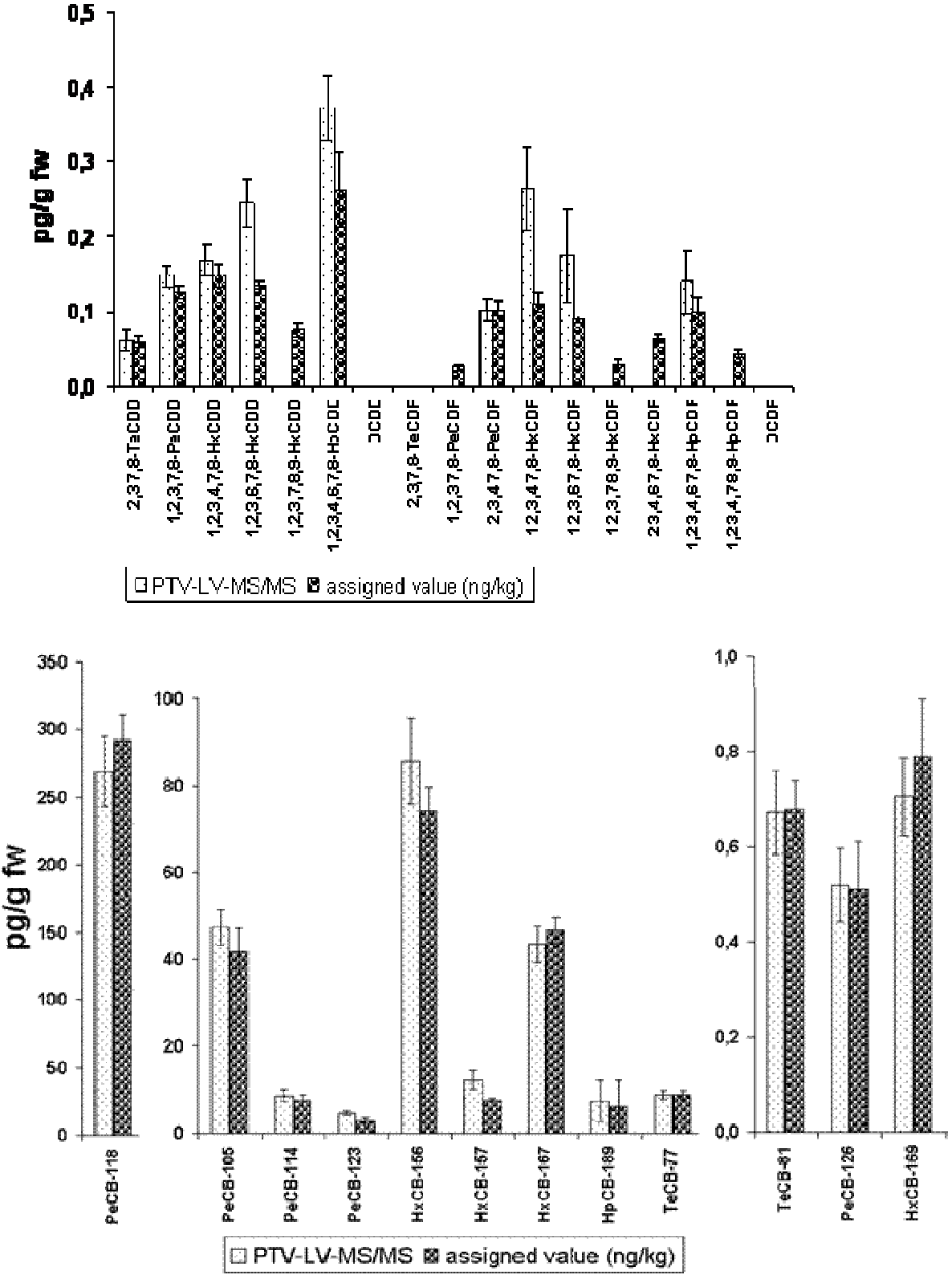

Figure 3-11 : Trueness assessment for PCDD/F and DL-PCB congeners in DIFF-02 pork tissue

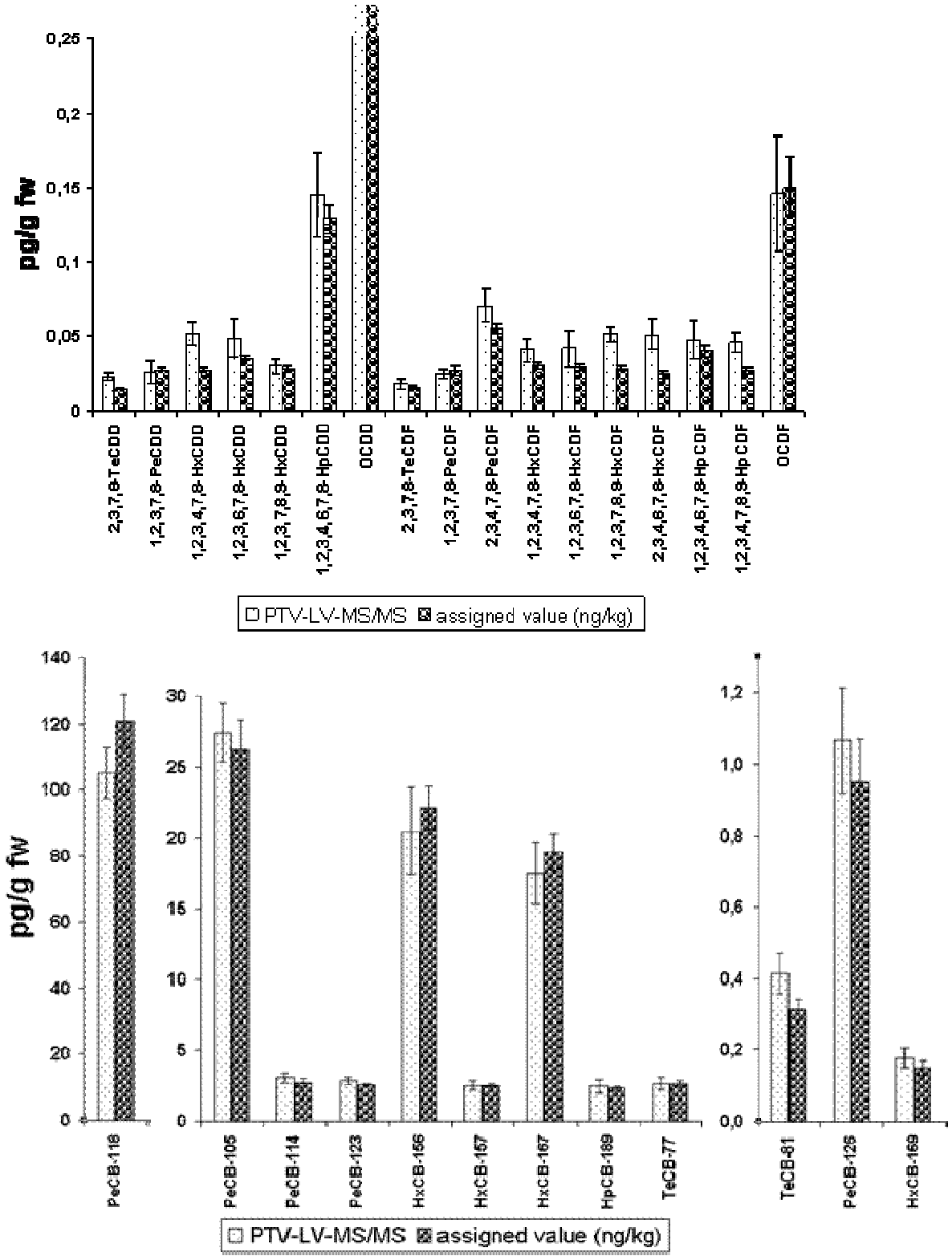

Figure 3-12: Trueness assessment for PCDD/F and DL-PCB congeners in DIFF-03 whole milk

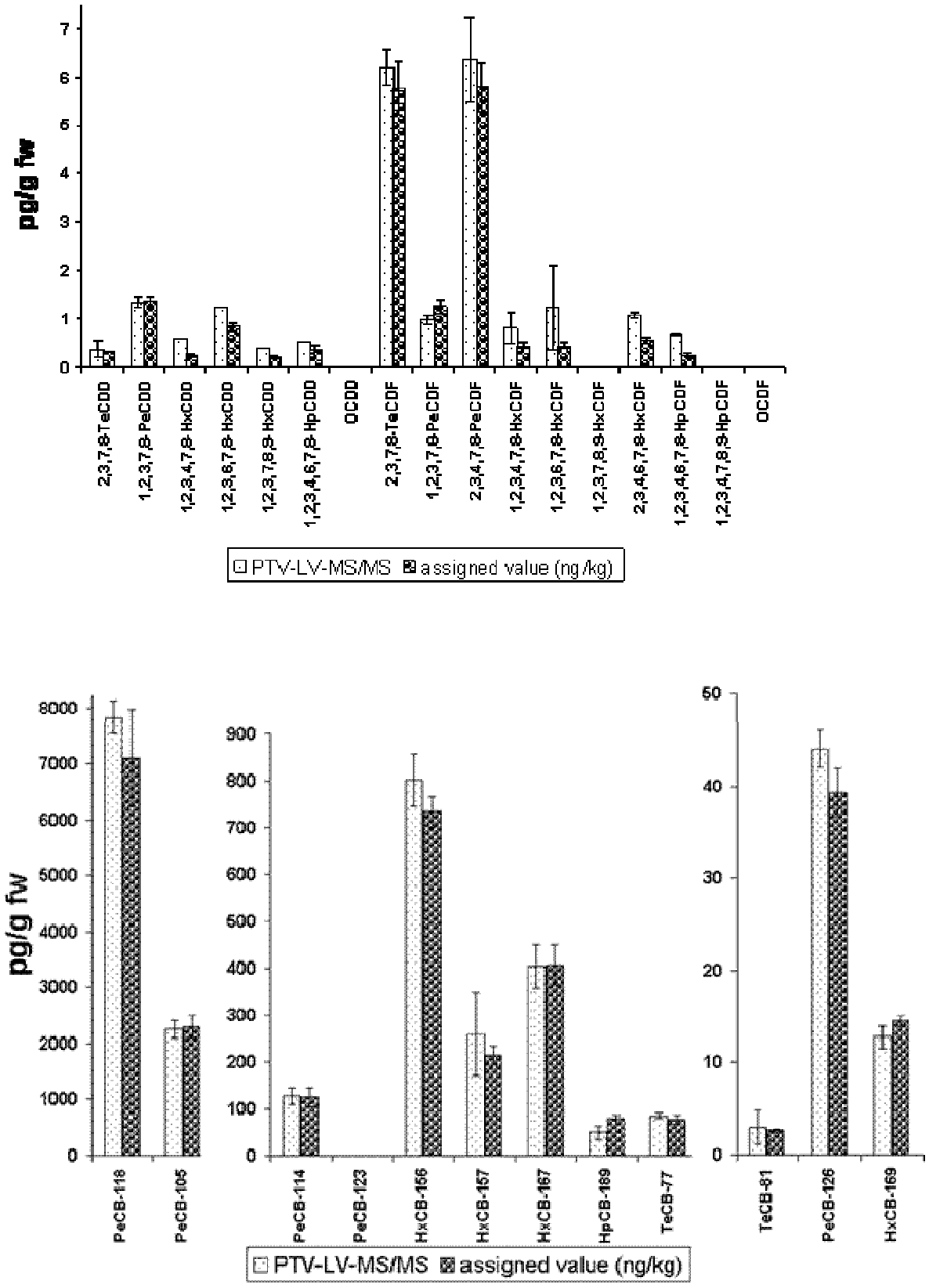

Figure 3-13: Trueness assessment for PCDD/F and DL-PCB congeners in DIFF-04 fish oil

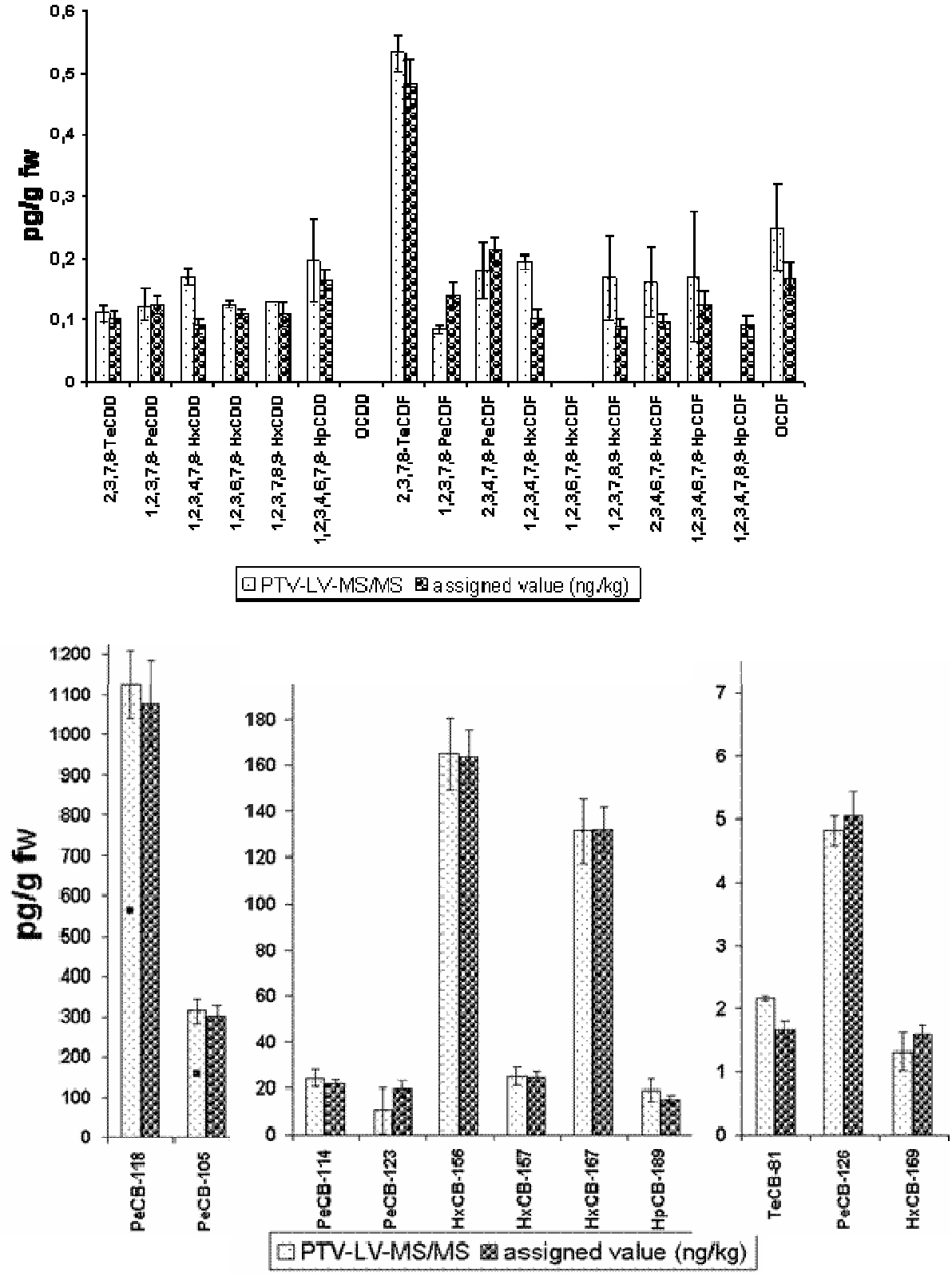

Figure 3-14: Trueness assessment for PCDD/F and DL-PCB congeners in DIFF-05 pig feed

Figure 3-10 to Figure 3-14 compare the reference value assigned with the HRMS method with the ones get by the alternative low resolution MS/MS method. All the 29 toxics congeners were analyzed. It appears that the PTV-LV-MS/MS performed similarly for the five investigated matrices independent of the levels. Even if there are discrepancies between mass fraction values for some congeners, especially for low PCDD/F levels, the congener profile is maintained. In pig tissue, whole milk and pig feed material, most of the PCDD/F concentrations were very close to the alternative method's LOQs. It yields to more variability in the results compared to HRMS results. Table 3-6 and Table 3-7 summarize TEQ values for the sum of the 17 PCDD/Fs and for the sum of the 29 congeners respectively. Compared to the previous results (see Figure 3-8), a small positive bias is observed with the alternative MS/MS method for all the matrices, excepted pig tissue (see Table 3-7). The rotation biases observed for the PCDD/F–TEQ and total TEQ are depicted on Figure 3-15 and Figure 3-16. The slopes of the regression lines are 0.93 and 0.92 respectively. The small biases are related to systematically slightly higher values for congeners with a high contribution to the TEQ. However, all the biases summarized in Tables 3-6 and 3-7, except one, comply with the trueness criteria for a confirmatory method in the Commission Regulation 1883/2006 (i.e. trueness : ± 20% for confirmatory GC/HRMS method). It can be concluded that the PTV-LV-GC-MS/MS method can yield very good PCDD/F-TEQ and total TEQ estimates at the concentration levels investigated. Tables 3-6 and 3-7 showed also that the within-lab reproducibility, expressed in % of RSD, matches the precision criteria of Commission Regulation 1883/2006 (i.e. intermediate precision < 30% for screening methods).

Table 3-6 : Synthesis of TEQ values for the 17 PCDD/F congeners

TEQ Σ PCDD/Fs	PTV-LV-MS/MS ng-WHO-TEQ/kg	HRMS ng-WHO-TEQ/kg	bias (%)	RSD* (%)
fish tissue	1.97	1.89	+ 4	6.5
pork tissue	0.296	0.307	- 4	29.2
whole milk	0.117	0.095	+ 24	14.8
fish oil	5.96	5.56	+ 7	6.8
pig feed	0.43	0.46	- 7	29.9

*: intermediate precision (within-laboratory reproducibility) of PTV-LV-MS/MS

Table 3-7 : Synthesis of TEQ values for the 29 toxic congeners

TEQ Σ PCDD/Fs, DL-PCBs	PTV-LV-MS/MS ng-WHO-TEQ/kg	HRMS ng-WHO-TEQ/kg	bias (%)	RSD* (%)
fish tissue	4.033	3.76	+ 7	4.6
pork tissue	0.442	0.446	- 1	20.5
whole milk	0.252	0.22	+ 15	8.2
fish oil	12.17	11.2	+ 9	3.9
pig feed	1.28	1.23	+ 4	12.5

*: intermediate precision (within-laboratory reproducibility) of PTV-LV-MS/MS

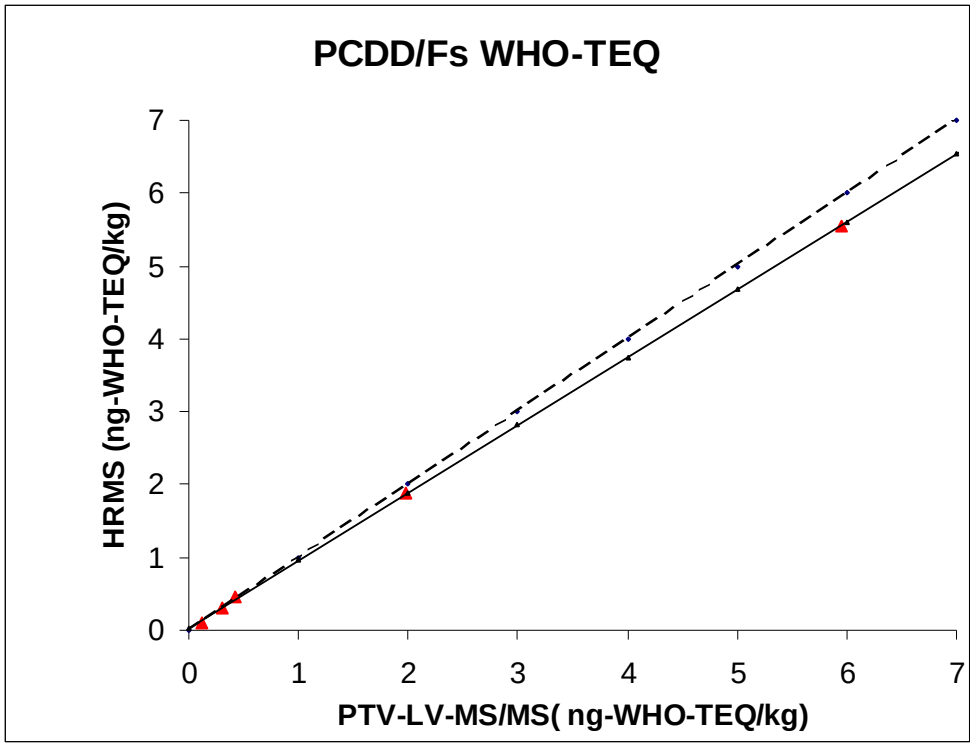

Figure 3-15: Representation of the functional relationships between the PTV-LV-GC/MS/MS method and the GC/HRMS method for the sum of the 17 PCDD/F congeners

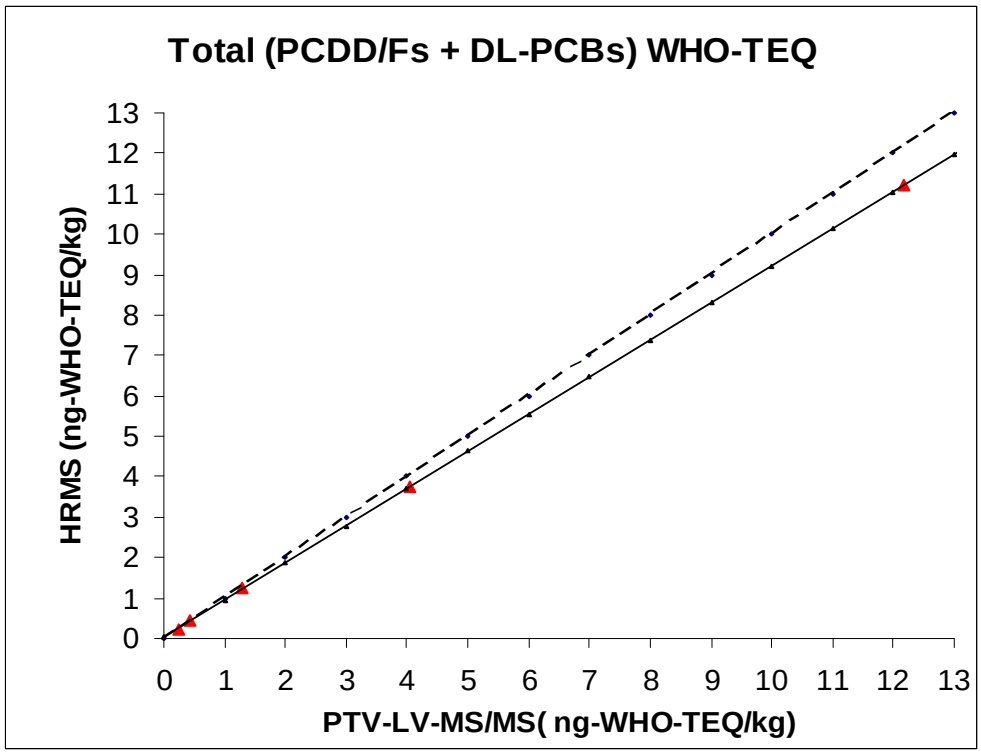

Figure 3-16 Representation of the functional relationships between the PTV-LV-MS/MS method and the GC/HRMS method for the sum of the 29 toxic congeners

3.4 Conclusions

The primary goal of the present study was to set-up a ^{13}C-labelled isotope dilution PTV-LV-GC-MS/MS method for the measurement of the 17 PCDD/Fs and the 12 DL-PCBs in foodstuffs. We demonstrated its feasibility. The method fulfils the European Regulation 1883/2006 analytical requirements regarding screening approaches. Consequently, PTV-LV-GC/MS/MS is an attractive technique and can be used as a cost effective complementary method to HRMS for dioxin levels monitoring in food and feed. Though the method can provide sufficiently accurate TEQ estimates, it may yield rather variable results for the mass fractions of individual congeners compared to GC/HRMS measurements. This is probably mainly related to higher limits of quantification but also related to the ion trap mass spectrometer itself which is much more influenced by sample extract quality compared to a HRMS instrument. The occurrence of matrix interfering compounds in final extracts may lead to dramatically reduce the sensitivity of target compounds by polluting the trap with matrix interfering ions. To achieve such accuracy during the feasibility study, special precautions for instrument maintenance (e.g. frequent cleaning of the ion volume) were essential to maintain the instrument at its maximum sensitivity performances, which may not be sustainable in routine practice.

In addition, data handling and processing time by the alternative approach for quantification by isotopic dilution need to be improved. For instance, quantification with well designed dioxin software by HRMS takes 7 minutes while it takes one hour by PTV-LV-GC/MS/MS. For these reasons, we cannot conclude that the method developed here is a screening method in terms of high throughput capacities. It is rather an alternative and complementary technique to HRMS.

Today, detection limits are low enough for most regulatory purposes, but may need to be pushed lower tomorrow due to the constant decrease of dioxin-like compounds in foodstuffs and also due to the pro-active EU approach that re-assesses periodically the maximum levels in order to bring human exposure below the tolerable weekly intake. Hence, analytical methodologies ought to be able to follow the trend. Even though the sensitivity of this technique is still slightly below the HRMS sensitivity, in the next few years ion trap MS/MS is expected to play an important role for the analysis of halogenated compounds. The coming years will show us how this alternative technique evolve and what place it comes to occupy in the measurement of dioxin and related compounds.