



Optimisation and use of tandem-in-time mass spectrometry in comparison with immunoassay and HRGC/HRMS for PCDD/F screening

J.-F. Focant ^{*}, G. Eppe, E. De Pauw

Mass Spectrometry Laboratory, Chemistry Department, University of Liege, Allée de la Chimie, 17, B6c Sart-Tilman, Liège B-4000, Belgium

Abstract

Rapid screening of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans using quadrupole ion storage tandem-in-time mass spectrometry (QISTMS) conjointly with polyclonal antibody immunoassay has been considered. The optimisation of the fragmentation of the parent ion in the trap has been completed. The analysis of fly ashes from a municipal waste incinerator contaminated at different levels has then been realised. Results obtained using QISTMS, HRMS and immunoassay are compared. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

During their lifetime, humans are exposed to a wide range of organic toxins in different amounts. Among the polyhalogenated aromatic family, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a class of non-volatile environmental contaminants that are not primary industrial products (Safe, 1986). These compounds (generally simply called “dioxins”) are formed as impurities in the manufacture of chemicals based on chlorophenols and chlorinated aromatic hydrocarbons (Cairns et al., 1980), as well as in various industrial processes such as wastes combustion, metal industry, wood burning... (Mukerjee, 1998). Once formed, dioxins are released into the environment and accumulate through the food chain up to humans. According to the number and position of the chlorine atoms, they are able to bind with the aryl

hydrocarbon (Ah) receptor, which gives them the possibility to exert a broad range of toxic and biological effects toward mammals (Tyler et al., 1998).

Since many years, the great environmental concern about the presence of these molecules at low levels (parts per trillion) in the environment has lead scientists to develop sensitive analytical methods. Powerful analytical tools as well as multiple-step isolation and cleanup procedures are necessary to determine trace levels of these analytes in complex contaminated samples. Due to its specificity and sensitivity, high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS) has become the reference method in this field. The usual procedure to quantify dioxins is based on isotopic dilution. This method rests on the measurement of two ions in the molecular cluster for each congener ($[M]^{+}$ and $[M + 2]^{+}$, for example) in comparison with their corresponding ^{13}C -labelled ions with verification of the isotopic ratios and retention times.

As HRGC/HRMS requires large investment and maintenance costs, alternative methods have been evaluated either to replace the reference method or at least

^{*} Corresponding author. Tel.: +32-4-366-3414; fax: +32-4-366-3413.

E-mail address: jf.focant@ulg.ac.be (J.-F. Focant).

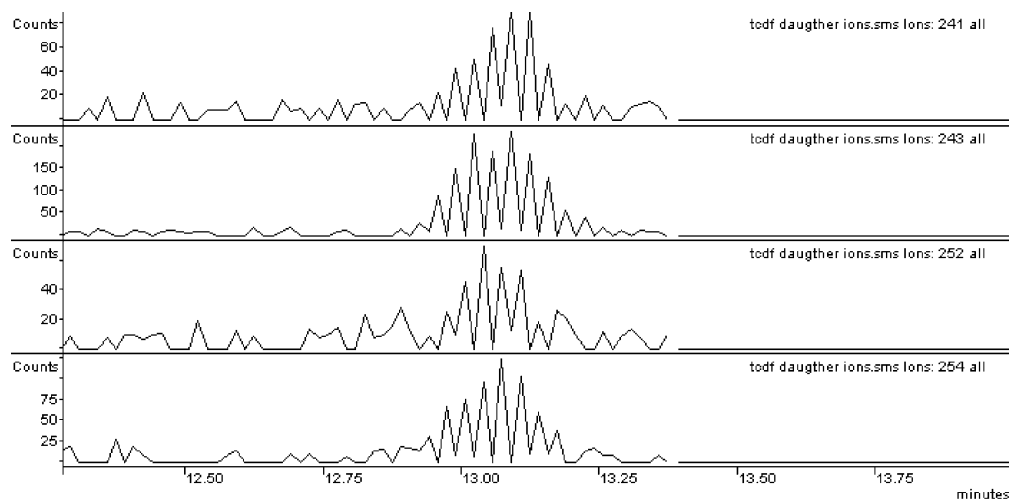


Fig. 1. Example of quantification for TCDF by MS/MS using isotopic dilution. This chromatogram represents native (241, 243) and labelled ^{13}C (252, 254) daughter ions.

to alleviate analysis costs by their use in preliminary screening. Only suspect samples are kept for analysis by the reference method. Capillary column gas chromatography/tandem mass spectrometry (GC/MS/MS) using a low-resolution quadrupole ion trap has been presented by Plomley et al. (1994) and Splendore et al. (1997) as a valuable screening method for PCDD/Fs. The storage of selected parent ions in the trap followed by their fragmentation by collision-induced dissociation (CID) yields characteristic daughter ions resulting from the loss of the COCl^{\cdot} fragment (Chess and Gross, 1980). The fragmentation of native and ^{13}C -labelled parent ions is monitored using multiple reaction monitoring (MRM). To ensure the production of two different daughter ions for each parent, selected parents ions have to contain at least one ^{37}Cl ; this is achieved by considering $[\text{M} + n]^+$ ($n = 2, 4, 6$) parent ions. The quantification is still carried out using the isotopic dilution technique, applied now on daughter ions instead of molecular ions (Fig. 1). The sensitivity reaches a level which, when coupled with the selectivity of the MS/MS method, can confer very good results in the evaluation of 2,3,7,8-substituted congeners' contribution to the toxic equivalency (TEQ) of the environmental samples.

In addition to the optimisation of an alternative mass spectrometry technique, the use and applicability of an immunoassay kit has been evaluated to demonstrate its performances when used together with MS/MS in screening campaigns. Among the different classes of existing dioxin assays (Bosveld and Vanden Berg, 1994; Garrison et al., 1996; Bovee et al., 1998; Schwirzer et al., 1998; Sugawara et al., 1998), a competitive inhibition enzyme immunoassay (EIA) based on a polyclonal antibody specific to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related congeners has been well described for soil

and fly ash analysis (Harrison and Carlson, 1997a,b, 1998). This commercially available EIA kit has a sensitivity reaching the picogram level. Its response is proportional to the toxic equivalency factors (TEFs) of the toxic isomers.¹

This paper illustrates the optimisation steps required for MS/MS and EIA. Results are compared with those obtained with the reference procedure. An analytical strategy involving a bioassay as screening test followed by good quantitation using GC/MS/MS is presented as a good approach for routine monitoring or for process optimisation.

2. Materials and methods

2.1. Chemicals

Toluene, hexane, methanol and water are Baker analysed HPLC reagents (J.T. Baker, Deventer, Netherlands). Dodecane was purchased from Merck-Schuchardt (Hohenbrunn, Germany). HCl (1 M) was prepared using HCl 92% purchased from Merck (Darmstadt, Germany) and fuming sulphuric acid prepared with H_2SO_4 95–97% Baker analysed reagents (J.T. Baker). Anhydrous Na_2SO_4 is a Baker analysed reagent (J.T. Baker) and was covered with toluene and left in an oven at 100°C overnight before use. The standard solution of 2,3,7,8-chloro-substituted $^{13}\text{C}_{12}$ -labelled dioxins (EPA 1613LCS), $^{12}\text{C}_{12}$ 1,2,3,4-TCDD as well as EIA

¹ High-Performance Dioxin/Furan Immunoassay Kit™, Cape Technologies, L.L.C., South Portland, USA.

calibration standards (DF1-CAL) were purchased from Campro Scientific (Veenendaal, Netherlands).

2.2. Instrumentation

All HRGC/HRMS analyses were performed using a VG-AutoSpec-Q high-resolution mass spectrometer (Fisons Instruments, Manchester, UK) and a 5890 Series II gas chromatograph (Hewlett–Packard, Wilmington, USA). Optimised GC conditions as well as operating mode of the mass spectrometer have been described elsewhere (Windal et al., 1998). Mainly, an SP2331 (60 m × 0.25 mm × 0.2 µm) capillary column (Supelco, Bellefonte, USA) was used in parallel with a minimum resolution of 10 000.

The GC/MS/MS analyses were carried out with a Saturn 2000 GC/MS/MS coupled with a Star 3400CX gas chromatograph and an 8200CX autosampler (Varian, Walnut Creek, USA). The Saturn 5.1 software version of the workstation was used. Mixtures were separated on a DB-5MS (30 m × 0.25 mm × 0.25 µm) capillary column (J&W Scientific, Folsom, USA). The GC conditions were optimised to separate 2,3,7,8-chloro-substituted congeners as follows: on-column injection of 1 µl of sample at 240°C, initial oven temperature of 150°C for 1 min, then increased at 30°C/min to 210°C, then increased at 1°C/min to 250°C and held for 7 min. A faster GC program has also been used during these experiments: on-column injection of 1 µl of sample at 240°C, initial oven temperature of 140°C for 2 min, then increased at 50°C/min to 220°C held for 2 min, then increased at 25°C/min to 250°C held for 11 min, then increased at 25°C/min to 300°C and held for 3.2 min. In both cases, He (N60, Air Liquide, France) was used as the carrier gas. The trap temperature was set at 200°C, with the transfer line at 250°C and a maximum number of 5000 ions in the trap.

2.3. Sample preparation

Experiments were carried out on fly ash samples collected by private industries at the bottom of electrostatic precipitators of Belgian municipal waste incinerators. Several types of samples, called A, R, L and T, containing different levels of PCDD/Fs, were analysed. All fly ash samples were pre-treated as described in a previous paper (Windal et al., 1998). Briefly, sufficient quantity of fly ash was stirred with 1 M HCl for 2 h and then washed several times with fresh water after the elimination of the acidic layer by centrifugation. Overnight-dried ashes were kept in a closed vessel. For the extraction of each sample, 1.5 g of ash were Soxhlet extracted for 20 h using 120 ml of toluene without any previous spike with labelled solutions. The extract was dried with sodium sulphate and filtered. The dried ex-

tract solution was then adjusted to 100 ml in a silanised volumetric flask and protected from light at 5°C until use.

Each immunoassay test was realised on exactly 2 ml of the 100 ml homogenised toluene solution to ensure that appropriate amount of the sample was in contact with the coated tubes later on and to allow the semi-quantitation of the analytes. These 2 ml aliquots were submitted to the required cleanup procedure described in the technical information manual provided with the kit (Cape Technologies, South Portland, USA). This cleanup included fuming sulphuric acid wash, hexane extraction and solvent exchange to methanol using Triton X-100 as keeper.

The remaining solutions contained in the volumetric flasks were spiked with 10 µl of ¹³C-labelled 2,3,7,8-dioxin solution to allow isotopic dilution quantification of the native dioxins by physico-chemical analysis. These solutions were concentrated using 50 µl of dodecane as keeper without any additional cleanup.

3. Results and discussion

3.1. MS/MS optimisation

The MS/MS method development was mainly divided into three steps. The first task was, as usual, to determine the retention time of the parent ions on the selected column. This began with the injection of a standard mixture containing the 16 2,3,7,8-PCDD/F congeners using full-scan mode. The elution pattern from 2,3,7,8-TCDF to OCDD using the 50 min GC program is illustrated in Fig. 2(a). This allowed slicing of the chromatogram into 10 different segments corresponding to each group of chlorination, with every one of these segments being intended to the MRM of the ¹²C and ¹³C coeluting congeners.

Due to the shift sometimes observed in retention times, when real samples are analysed, a separation between PCDD and PCDF runs was considered. The disadvantage of the two separate injections was partly compensated by the possibility of using a faster method, which included only four or five segments with a total time of 23 min, as illustrated in Fig. 2(b).

The second task concerned the determination of the best conditions to efficiently ionise and trap the parent ions of each congener. Default parameters for the preliminary step of isolation of the ions were used; all the attention was focused on the excitation step yielding the daughter ions.

The CID parameters were then optimised. The main parameters which could be optimised during the excitation step were the excitation mode, the CID time, the excitation voltage and the stability parameter q_z issued

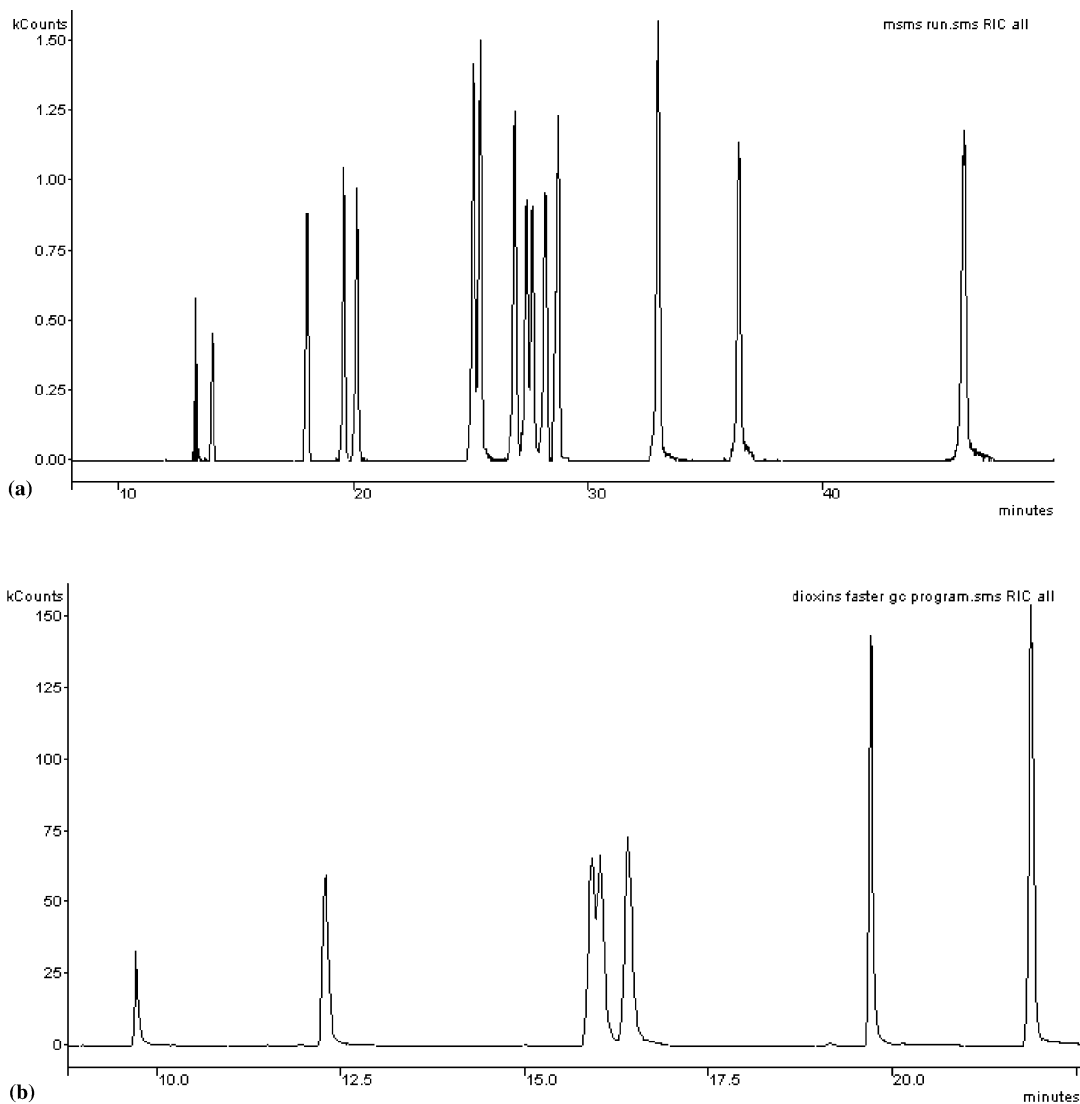


Fig. 2. Example chromatograms corresponding to: (a) 50 min GC program, PCDD and PCDF congeners; (b) 23 min GC program, 7 PCDDs only.

of the Mathieu second-order differential equation which accounted for the ion motion in the trap (March, 1991). The last two parameters were closely linked and an optimum of the couple (CID voltage, q_z) had to be found for each 2,3,7,8-congener.

Two methods are available to increase the vibrational energy of the parent ion to allow its fragmentation at sufficient rate: resonant and non-resonant excitation. Firstly, a comparison between these two modes was carried out. Most of the MS/MS experiments described in the literature use resonant excitation mode. This method consists in application of an additional high-frequency dipole field to the end cap electrodes of the ion trap. The frequency is chosen in order to match the secular frequency of the trapped ion, resulting in an

increase of the kinetic energy of the ion. Kinetic energy is transformed into internal energy upon collisions with the helium present in the trap. The internal energy and the residence time are sufficient to allow fragmentation reactions involving rearrangements. The non-resonant mode is suitable for single-bond cleavage. An additional drawback of non-resonant excitation is that it suffers the loss of ions by ejection when slow fragmentation reactions (rearrangements) are monitored. Considering that the loss of COCl^+ for PCDD/Fs occurs via the breakage of multiple chemical bonds, better results were expected using the resonant mode.

Regarding the couple (CID voltage, q_z), the stability parameter was first tuned to modify the position of the ion within the stability diagram of the trap. With the

software used, q_z could be translated in term of excitation storage level (CID rf). This parameter was linked with the broadband multi-frequency waveform used during the isolation of the parent ion.

In order to realise the first selection of the q_z value without using runs that were too long, 1,2,3,4-TCDD was used rather than a mixture of all congeners. MS/MS optimisation was performed on the molecular ion of TCDD (m/z 334), and the q_z value varied in the range of 0.2–0.5. A low CID voltage (0.2 V) was applied to avoid fragmentation. This indicated a q_z value of 0.3 as corresponding to the maximal intensity of the m/z 334 ion.

Using this $q_z = 0.3$ value as starting point, the determination of the optimum voltage for resonant CID excitation was done with the automated methods development (AMD) tool. This consisted of performing the maximum number of cycles through the different voltages across each peak. AMD allowed the CID voltage to be incremented on a scan-by-scan basis for up to 10 scans per cycle. In our case, values from 0.8 to 1.5 V by increments of 0.2 V were studied. The optimum voltage for maximum intensity of 1,2,3,4-TCDD daughter ion formation (m/z 268 and 270) was around 1.3 V, as illustrated on Fig. 3. It is interesting to note that isotopic ratios between m/z 268 and 270 ions for

this CID amplitude (0.33 V) as well as for higher intensity areas at other voltages (0.34, 0.32 V) are very close to the theoretical value (0.33 V).

Extrapolation of the 1.3 V set to the entire group of ^{12}C 2,3,7,8-PCDD/Fs and q_z optimisation were then done following some direct modifications. First, the scan time was decreased to 0.3 s/scan to ensure that the system performed enough scans for each fragmentation reaction of the AMD performed on q_z across the analytical peak. Second, excitation amplitudes for PCDFs were set to greater values due to the higher activation energy required to effect the loss of COCl from PCDFs relative to PCDDs (Plomley and March, 1996). Due to this higher stability, one could often observe the remaining presence of parent ions for PCDFs while PCDD parent ions were absent.

Finally, CID time was optimised. It was found for these chlorinated compounds that a short CID time of 5 ms gave a better signal–noise ratio than the usual longer ones. This feature allowed the collection of more scans across GC peaks, reducing the scan time.

A last parameter which was peculiar to the resonant mode was the number of frequencies applied in the excitation wave form (CID bandwidth). Changing the CID bandwidth permitted simultaneous excitation of ions

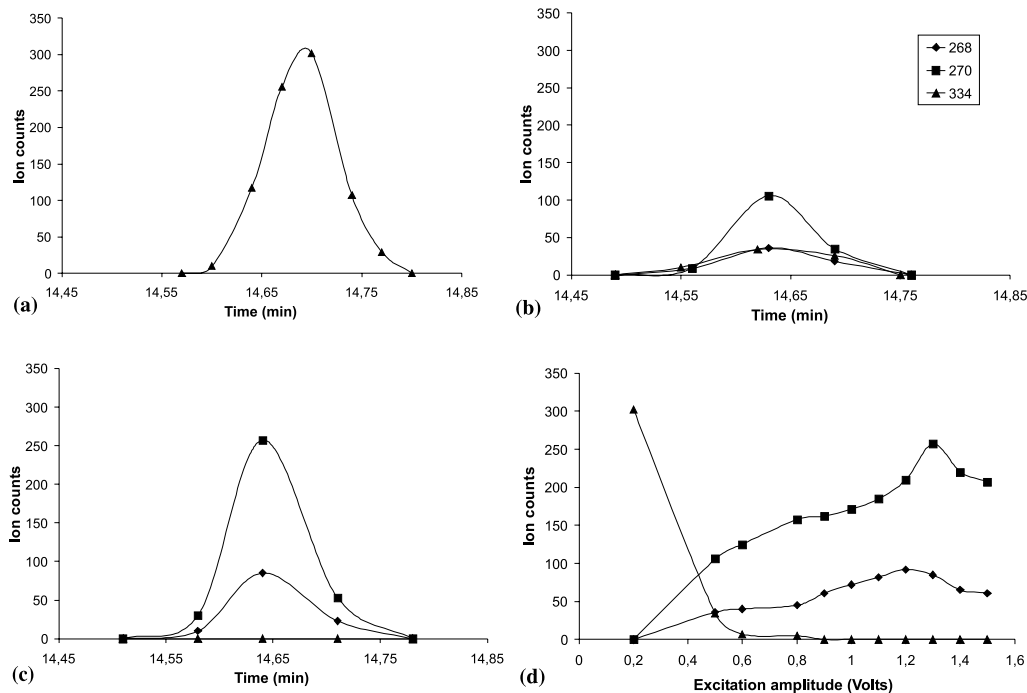


Fig. 3. Fragmentation curves for resonant excitation of 1,2,3,4-TCDD with a q_z value of 0.3: (a) Using a CID voltage of 0.2 V, the parent ion m/z 334 is not fragmented and daughter ions are not formed yet; (b) Increasing the CID amplitude to 0.5 V gives enough energy for the effective collision of the parent ion with the He and yield to the m/z 334 ion intensity due to the formation of daughter ions m/z 268 and 270; (c) If voltage is set to 1.3 V, the maximal intensity for daughter ions is achieved without any remaining parent ion; (d) Global evolution of the species following CID voltage.

Table 1
Optimised parameters for the MS/MS analysis of 2,3,7,8-PCDD/Fs

Segment #	Congeners	Molecular ions (<i>m/z</i>)	CID amplitude (V)	CID rf (<i>m/z</i>)	Daughter ions (<i>m/z</i>)	Isotopic ratios	Validity ($\pm 20\%$)
Dioxin							
1	TCDD ¹² C	321.89 (M+2)	1.3	106	257/259	0.33	0.26 < 0.33 < 0.4
	TCDD ¹³ C	333.93 (M+2)	1.3	147	268/270	0.33	0.26 < 0.33 < 0.4
2	PeCDD ¹² C	357.85 (M+4)	1.3	158	293/295	0.66	0.53 < 0.66 < 0.80
	PeCDD ¹³ C	369.89 (M+4)	1.3	163	304/306	0.66	0.53 < 0.66 < 0.80
3	HhxCDD ¹² C	391.81 (M+4)	1.3	173	327/329	0.5	0.40 < 0.50 < 0.60
	HxCDD ¹³ C	403.86 (M+4)	1.3	178	338/340	0.5	0.40 < 0.50 < 0.60
4	HpCDD ¹² C	425.77 (M+4)	1.5	188	361/363	0.4	0.32 < 0.40 < 0.48
	HpCDD ¹³ C	437.81 (M+4)	1.5	193	372/374	0.4	0.32 < 0.40 < 0.48
5	OCDD ¹² C	461.73 (M+6)	1.5	204	397/399	0.6	0.48 < 0.60 < 0.72
	OCDD ¹³ C	473.77 (M+6)	1.5	236	408/410	0.6	0.48 < 0.60 < 0.72
Furan							
1	TCDF ¹² C	305.89 (M+2)	1.6	135	241/243	0.33	0.26 < 0.33 < 0.4
	TCDF ¹³ C	317.94 (M+2)	1.6	140	252/254	0.33	0.26 < 0.33 < 0.4
2	PeCDF ¹² C	341.86 (M+4)	1.6	150	277/279	0.66	0.53 < 0.66 < 0.80
	PeCDF ¹³ C	353.90 (M+4)	1.6	175	288/290	0.66	0.53 < 0.66 < 0.80
3	HxCDF ¹² C	375.82 (M+4)	2	207	311/313	0.5	0.40 < 0.50 < 0.60
	HxCDF ¹³ C	387.86 (M+4)	2	171	322/324	0.5	0.40 < 0.50 < 0.60
4	HpCDF ¹² C	409.79 (M+4)	2	203	345/347	0.4	0.32 < 0.40 < 0.48
	HpCDF ¹³ C	421.82 (M+4)	2	209	356/358	0.4	0.32 < 0.40 < 0.48

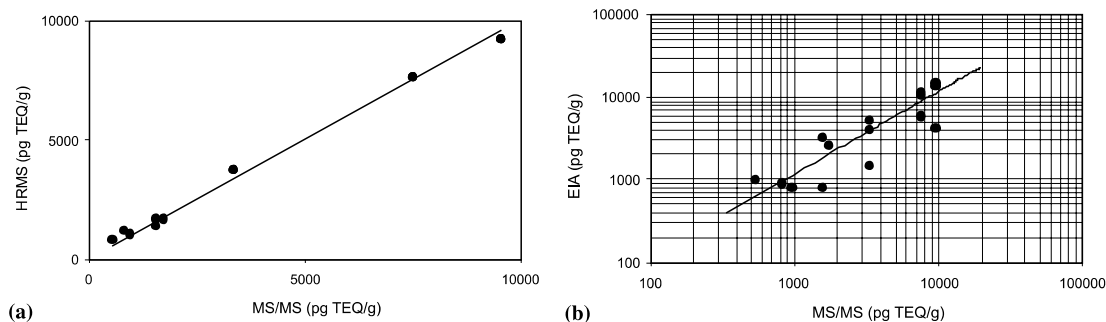


Fig. 4. Analysis of real fly ash samples: (a) Correlation curve between MS/MS and HRMS measurements; (b) EIA response against MS/MS results.

within a range of masses. It was modified up to seven frequencies, but the main effect was the need of increasing the CID amplitude to compensate the spread of the energy on more frequencies.

The CID conditions were optimised for all natives and isotopic labelled 2,3,7,8-PCDD/Fs, and a multi-segment method to acquire the data was built (see Table 1).

Using these parameters on real fly ash samples, a good correlation (correlation coefficient of 0.99, slope of 1.01) between MS/MS and HRMS could be observed, as clearly illustrated in Fig. 4(a). The MS/MS measurements showed a relative standard deviation (RSD) of about 10–15% for total TEQ values, while RSD for HRMS quantification was between 5% and 10% depending on the nature of the sample contamination level. The RSD in MS/MS was generally greater for lower chlorination degree congeners which were often present in smaller amounts. The limits of detection using MRM applied on two ions in each segment were close to 1 pg/ μ l for TCDD and 3 pg/ μ l for OCDD.

3.2. Immunoassay semi-quantification

To allow comparison between the biological method and mass spectrometry results, a calibration curve has been calculated for each EIA run used for quantitative analysis. The complete description of the use of negative control, calibration standards and optical density values interpretation can be obtained in the calculation module C worksheet available for download from the Cape web site.²

Fig. 4(b) shows the MS/MS measurements based on calculation of each 2,3,7,8-congener contribution regarding its TEF. These TEFs are plotted versus the EIA response also expressed in picogram TEQ per gram of sample. This shows that the EIA response follows quite

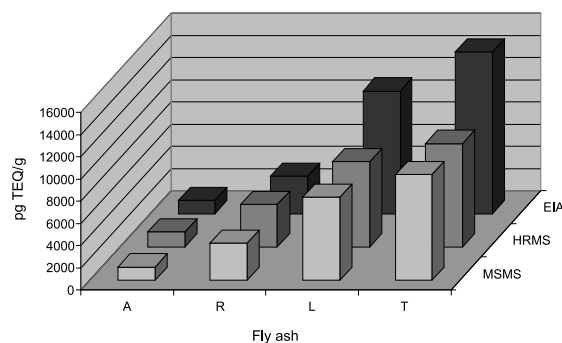


Fig. 5. Overview of the measurements on the different fly ashes.

well the evolution in the PCDD/F concentrations. The relatively good correlation (correlation coefficient of 0.84, slope 1.19) observed reflects the good relation between the WHO TEFs used and the cross reactivities of the EIA. The disparities previously reported (Zennegg et al., 1998) between I-TEFs and cross reactivities are lowered using WHO TEFs.

The profile of Fig. 5 indicates that the EIA generally provides a general overestimation of the PCDD/F contents. This means that the probability of finding false positive results is possible, but on the other hand, it gives a safety margin for the biological screening.

4. Conclusion

A simple and time-effective analytical strategy for the monitoring of the PCDD/Fs' TEQ content in a large number of samples has been designed and verified. It includes an immunoassay for a first sorting out and GC/MS/MS. This approach should meet the need for tools for extensive monitoring.

Positive samples to EIA (above a threshold value) are quantified by GC/MS/MS. A fully secure strategy should include the GC/MS/MS quantitation of a

² Cape Technologies web site: <http://www.cape-tech.com/>.

random selection of negative EIA samples. Random selection of positive samples can be, when required, confirmed by HRGC/HRMS. This combination of immunoassay and physico-chemical methods will reduce the global time and cost of efficient monitoring. Current work aims to decrease the detection limits of these alternative methods to make them suitable for the analysis of biological samples (Hayward et al., 1999).

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