Rapid Screening of PCDD/Fs : Comparison between Immunoassay, GC/MS/MS and HRGC/HRMS on different fly ashes.

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F's) represent a class of non-volatile halogenated aromatic environmental contaminants that have been of interest since many years (1). Their ability to bind the Aryl Hydrocarbon Receptor (AhR) has made them one of the most studied endocrine disruptor group of compounds (2). Powerful analytical methods are usually necessary to determine the trace levels of this class of analytes in contaminated samples. High Resolution Gas Chromatography coupled with High Resolution Mass Spectrometry (HRGC/HRMS) is the only reference method which combines the required specificity and sensitivity. It is expensive, time consuming and requires high capital cost equipment. The need of alternative procedures has forced scientists to find cheaper and faster methods but still sensitive in the parts-per-trillion range.
Capillary column Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS) using low resolution quadrupole ion trap has been presented by March (3) as a valuable screening method for PCDD/Fs. The advantage of ion trap analyser is to be able to accumulate a selected ion species while other ion species are ejected from the trap in a resonant mode. The collision-induced-dissociation (CID) of selected parents ions can then selectively yield to the formation of characteristic daughter ions. This permits to increase the sensitivity which, when coupled with the selectivity of the method, can give very good results in the evaluation of congeners contribution to the Toxic Equivalency (TEQ) of the samples.
Immunoassays also appeared to have possible applications in this field. Several types of them have been presented since few years (4,5,6,7). Among the different class of these assays, a competitive inhibition Enzyme Immunoassay (EIA) based on polyclonal antibody specific to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related congeners has been well described (8). A commercially available EIA kit with picogram sensitivity and PCDD/Fs toxic isomers specificity in correlation with their Toxic Equivalency Factors (TEFs) has been used (9).

This study focused on the comparison between these alternative methods and the classic one to demonstrate their complementarity in screening campaigns.

Materials and methods

Experiments were carried out on fly ash (A, R, L, T) from municipal waste incinerators containing different levels of PCDD/Fs. Acidic pre-treatment of fly ash samples has been exposed in a previous paper (10). Extracted samples were directly analysed using mass spectrometry without any additional cleanup provided.

HRGC/HRMS analysis were performed using a VG-AutoSpec-Q high-resolution mass spectrometer (Fisons Instruments, Manchester, UK) and a Hewlett-Packard (USA) 5890 Series II gas chromatograph equipped with a SP2331 (60m x 0.25mm x 0.2µm) capillary column (Supelco, USA). The operating mode of the mass spectrometer and the GC conditions have been described else where (10).

GC/MS/MS analysis was carried out with a Saturn 2000 GC/MS/MS coupled with a Star 3400CX gas chromatograph and a 8200CX autosampler (Varian, USA). Mixtures were separated on a DB-5MS (30m x 0.25mm x 0.25µm) capillary column (Alltech, USA). Loss of $COCl^{\bullet}$ from selected parent ions was monitored using optimised parameters concerning voltage determining the amplitude of ions oscillations (CID), frequency dissociation of the selected ions and the duration of dissociation process.

The sample preparation method and the calculation modules for the semi-quantitative interpretation of results from the immunoassay developed by Cape Technologies (South Portland, USA) can be found in the technical information manual provided with the kit.

Results and Discussion

Fly ash samples were soxhlet extracted using toluene. Aliquots of extraction mixture were then separated from the main solution for a solvent exchange before the analysis by EIA. The main solution was spiked with labelled compounds (^{13}C) at this stage (isotope dilution, EPA 8280 method) and concentrated in dodecane before chemical analysis.

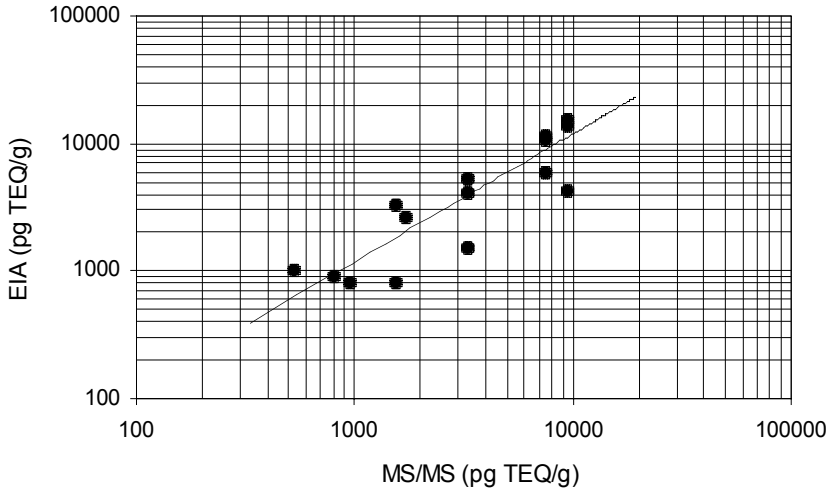

Fig. 1 : EIA response against MS/MS analysis of fly ash samples

Figure 1 shows the MS/MS measurements based on calculation of each 2,3,7,8-congener contribution regarding their WHO TEF. These are plotted versus the EIA response also expressed in pg TEQ/g of sample. The correlation coefficient for this plot is 0.84 with a slope of 1.19. This shows that EIA response follow quite well the evolution in the PCDD/Fs concentration.
The very good correlation (correlation coefficient of 0.99, slope of 1.01) between MS/MS and HRMS is clearly illustrated in figure 2.

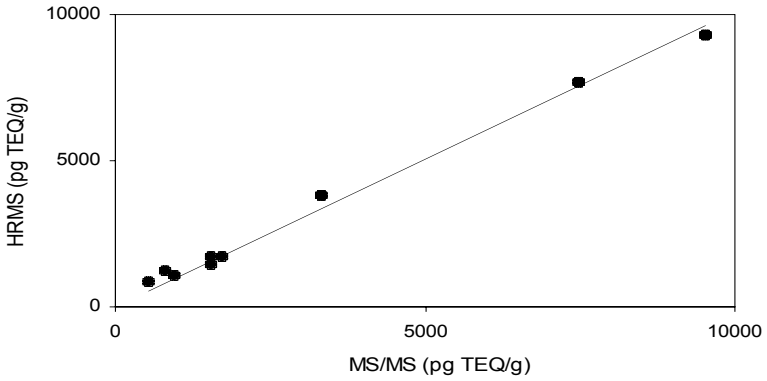

Fig. 2 : Correlation between MS/MS and HRMS analysis of fly ash samples

MS/MS measurements show a relative standard deviation (RSD) of about 10 to 15% while RSD for HRMS quantifications is between 5 to 10% dependent on the nature of the sample contamination level.

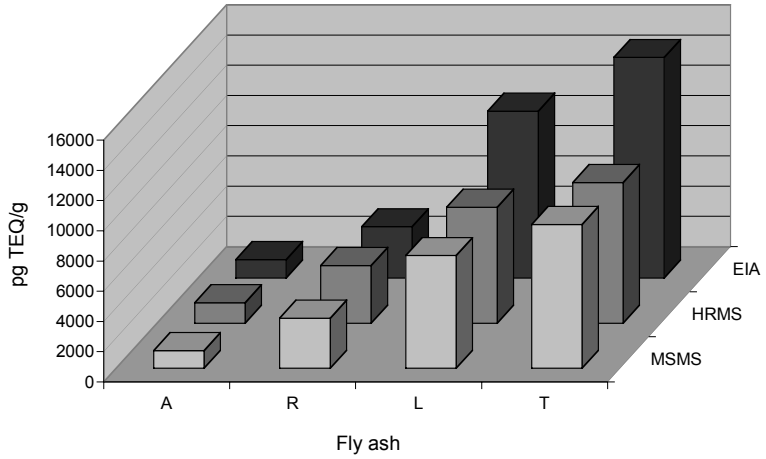

Fig. 3 : Global profile of the comparison between EIA, MS/MS and HRMS.

The profile of figure 3 indicates that the EIA provides a general overestimation of the PCDD/Fs contents. This fact means that false positives samples could be found but, in an other hand, it gives a safety margin for the biological screening.

The preliminary results indicate that the immunoassay can be used for a first sorting out in the monitoring of a large number samples. It yields a good appreciation of the global content in PCDD/Fs. Randomly selected negative samples can easily and cost-effectively be confirmed using a simple GC/MS/MS apparatus to achieve full confidence in the method whereas positive samples can be, when required, confirmed by HRGC/HRMS. A reference method such as HRGC/HRMS has still to be used to distinctly assess the TEQ values of individual congeners in positive samples. This combination of immunoassay and physico-chemical analysis should reduce global time for risk assessment of large number environmental samples.

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