

# GAS CHROMATOGRAPHY – ATMOSPHERIC PRESSURE PHOTOIONIZATION – HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF PCDD/Fs IN ENVIRONMENTAL AND FOOD SAMPLES

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## Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) constitute an important group of ubiquitous pollutants of great concern due to their high toxicity and persistence in the environment. PCDD/Fs have never been deliberately produced but they have been released in the environment as byproducts from combustion processes and industrial synthesis of other chlorinated chemicals. Although their emissions in most countries have significantly decreased as direct consequence of less contaminating industrial processes<sup>1</sup> and stricter environmental regulations<sup>2</sup>, their presence in the environment and food continues being a subject of great interest. PCDD/Fs analysis requires an extensive and time-consuming sample clean up in order to remove potential interferences<sup>3</sup>. Among all the developed methodologies, gas chromatography coupled to high resolution mass spectrometry (HRMS) using double-focusing analysers is the reference technique for PCDD/Fs analysis. This method offers the required sensitivity and selectivity for their determination in food and environmental samples around the femtogram level, removing the contribution to the response of potential interfering halogenated pollutants<sup>4</sup>. In the recent years, the use of Orbitrap high resolution mass analyzers, have demonstrated their capability in the analysis of environmental pollutants, showing a high selectivity and sensitivity at a very high resolving power (70,000–120,000). In addition, GC-MS analysis has been moving to the use of atmospheric pressure ionization (API) techniques. Thus, atmospheric pressure chemical ionization (APCI) and photoionization (APPI) sources have been introduced in the laboratories opening a new field of applications, since they could ionize a wider range of compounds than classical ionization techniques and offer a soft ionization that make possible to reduce the fragmentation of the molecular ion<sup>5</sup>. Concerning the GC-APPI source, it has been developed for their coupling to Orbitrap-high resolution mass spectrometers that guarantees the resolving power required and also allows the acquisition in full-scan mode at high sensitivity, becoming a potential useful tool for the analysis of halogenated pollutants.

In this work, the capabilities of the GC-APPI-HRMS have been evaluated for the analysis of PCDD/Fs in food and environmental samples. In addition, a critical comparison about the results obtained using GC-APPI-HRMS method was performed using selected samples with a wide range of concentration levels. The results and conclusions of the evaluation study are presented here.

## Materials and methods

A set of six calibration standard solutions (CSL-CS4) of the 17 regulated 2,3,7,8-chloro-substituted PCDD/Fs containing the corresponding <sup>13</sup>C<sub>12</sub>-labeled compounds in nonane, EPA-1613 CVS, were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada). The GC-APPI-HRMS analysis was performed using a Trace 1300 gas chromatograph, equipped with a AS 1310 autosampler and coupled to a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The ionization source was an atmospheric pressure photoionization source for gas chromatography (GC-APPI) (MasCom Technologies GmbH, Bremen, Germany) operating in negative-ion mode. A DB-5ms UI GC fused-capillary column of 60 m x 0.25 mm I.D. and 0.25 µm of film thickness (Agilent Technologies, Santa Clara, CA, United States), was employed for the chromatographic separation of the analytes. Helium was used as carried gas at constant flow of 1.1 mL min<sup>-1</sup>. Injector temperature was kept at 280 °C and samples and standards were injected (1.5 µL) in the splitless injection mode (1 min). The oven temperature was programmed from 140 °C (1 min) to 200 °C at 20 °C min<sup>-1</sup>, and then to 300 °C at 2 °C min<sup>-1</sup> (held 15 min). The transfer line, source and capillary temperatures were set at 280 °C, 250 °C and 225 °C, respectively. The GC-APPI source was equipped with a 10.6 eV krypton lamp (Syagen, Santa Ana, CA, USA) using nitrogen as make-up gas (gas pressure of 4 a.u.) and benzene vapours (90 µL min<sup>-1</sup>) as dopant. Data was acquired in full-scan mode (*m/z* 100 – 600) at a mass resolution of 35,000 (FWHM, at *m/z* 200), while maximum injection time and AGC were set at 100 ms and 3·10<sup>6</sup>, respectively. Quantification was performed by

isotope dilution using relative response factors (RRFs). Xcalibur v 3.1 software was used for controlling the instrument setup and processing the data acquisition.

Analyses of food and environmental samples were based on validated methodologies developed at the Laboratory of Dioxins (IDAEA, CSIC, Barcelona, Spain). It consisted in a Soxhlet extraction of samples with toluene for 24 h, and purification of the extracts by automated clean-up on the Power-PrepTM System (Fluid Management Systems, Inc., Waltham, M.A., USA), which includes a sequential use of multilayer silica, basic alumina and carbon adsorbents, prepackaged in disposable Teflon columns and hermetically sealed (Fluid Management Systems, Waltham, M.A., USA). The determination of PCDD/Fs by GC-EI-MS was performed on a 6890N Network GC System Agilent gas chromatograph (Agilent Technologies Inc., Palo Alto, USA) fitted with a DB-5ms UI fused silica column (J&W Scientific) and connected through a heated transfer line kept at 280 °C to an AutoSpec Ultima NT high resolution mass spectrometer (double focusing mass analyzer, EBE geometry, Waters, Manchester, UK). MS conditions were: electron ionization (EI+, 32 eV) mode using a source temperature of 275°C and operating in selected ion monitoring (SIM) mode at a resolution of 10,000 (10% valley definition).

## Results and discussion:

### *GC-APPI ionization of PCDD/Fs*

The ionization behavior of PCDD/Fs was evaluated in positive and negative-ion modes using both direct photoionization and dopant-assisted photoionization using vapors of different gas dopants. Operating in positive-ion mode, all the analytes yielded the  $[M]^{+}$  molecular ions (Figure 1, up-left and right) and their abundances decreased when increasing the chlorine substituents.

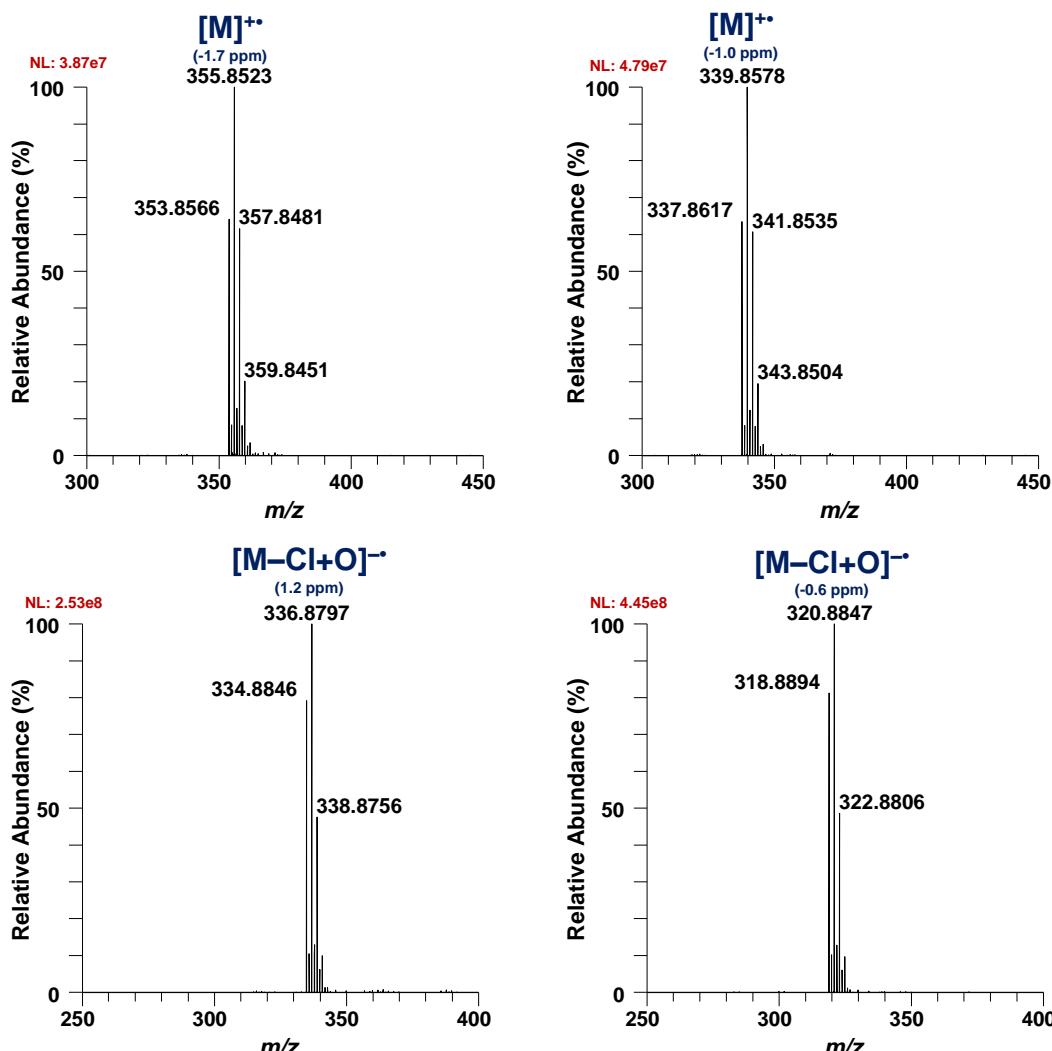


Figure 1. Positive-ion and negative-ion APPI full-scan mass spectra of 1,2,3,7,8-PeCDD (up and bottom left) and 1,2,3,7,8-PeCDF (up and bottom right).

It could be also observed that the ionization efficiency of the analytes increased using dopants that were photo-ionized generating their own molecular ion, such as chlorobenzene, toluene or benzene (Fig. 2a). In contrast, those dopants that generated the protonated molecule, such as acetone and tetrahydrofuran (Fig. 2b), showed lower responses for PCDDs and PCDFs since the charge-exchange reactions were not favored (Fig. 2c). Concerning negative-ion APPI, the ionization was generally mediated by the superoxide ion (Fig. 2d), which could capture the electrons released after dopant photoionization. PCDFs were ionized by the formation of a characteristic phenoxide ion (Fig. 2e) as it can be observed in Figure 1 (down-right). In the case of PCDDs, all of them also generated the  $[M-Cl+O]^-$  (Figure 1, down-left), although low and highly chlorinated PCCDs showed others intense ions corresponding with the loss of a chloride atom (Fig. 2f) that can be combined with the attachment of the superoxide ion (Fig. 2g).

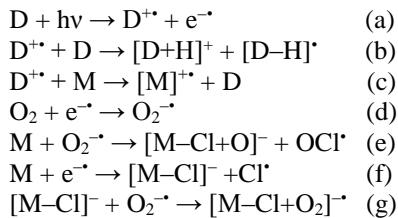


Figure 2. Ionization reaction in positive and negative-APPI mode (D: dopant).

Generally, the highest ionization efficiency was achieved in the negative-ion mode due to the high electronegativity of these compounds. Among the dopant tested (acetone, toluene, chlorobenzene, diethyl ether, benzene and tetrahydrofuran) those that presented the highest vapor pressures (acetone and diethyl ether) provided higher responses, since they allowed a higher concentration of electrons in the gas-phase. Moreover, benzene also showed a high ionization efficiency for PCDD/Fs, especially for the TCDD, although it has a lower vapor pressure than acetone and diethyl ether. Therefore, benzene was selected as the most suitable dopant since it allowed a high ionization efficiency for all the compounds. Comparing the capabilities of the two ionization modes, negative-ion APPI was selected as the most suitable method to achieve maximum sensitivity on the PCDD/F determination.

#### *Performance of the GC-APPI-HRMS method*

Quality parameters of the GC-APPI-HRMS method, such as linearity, limits of detection, and run-to-run precision, were established using standard solutions. Linearity of the target compounds was studied by analyzing six standard solutions (CSL-CS4) in a range of 0.1-40 pg  $\mu L^{-1}$  (for TeCDD and TeCDF), 0.5-200 pg  $\mu L^{-1}$  (from PeCDD/Fs to HpCDD/Fs) and 1.0-400 pg  $\mu L^{-1}$  (for OCDD and OCDF). For all the compounds, the determination coefficient ( $R^2$ ) of the calibration curves were higher than 0.9997 and the RRF values were always reproducible through a long period. Limits of detection (LODs) were estimated experimentally injecting standard solutions at low concentration levels. LODs for most of the analytes ranged from 0.5 to 8 fg injected, except for TCDD, which was 80 fg injected, demonstrating the high detection capability of the GC-APPI (-)-HRMS method. These instrumental LODs were good enough for the sensitive detection of all the compounds in real samples. Run-to-run precision was also evaluated along the calibration range obtaining relative standard deviation values (RSD, %) lower than 10%.

#### *Analysis of food and environmental samples*

In order to examine in depth, the feasibility of the proposed method for the determination of PCDD/Fs, it was applied to the analysis of food and environmental samples. Firstly, two certified reference materials (CRMs) consisting on a sludge (BCR-677) and a fly ash (BCR-615) were analyzed to demonstrate the validity of the developed method in environmental samples. For this purpose, extracts of the certified reference materials were analyzed by the proposed GC-APPI-HRMS method and the reference GC-EI-HRMS method with a double-focusing mass analyzer. Generally, good agreement between two techniques and the certified reference values was achieved for all compounds with differences in the concentrations lower than 10%. Figure 3 shows the comparison on the concentrations obtained by both GC-APPI-HRMS and GC-EI-HRMS techniques as well as the certified values.

To assess the applicability of the GC-APPI-HRMS, the method was applied to the analysis of selected food (feed, feed oil and soybean meal) and environmental (sediments) at concentrations ranged from 0.08 to 100 pg g<sup>-1</sup>. The results obtained were comparable with those obtained by GC-EI-HRMS demonstrating the capability of the GC-APPI source for the analysis of PCDDs and PCDFs at ultra-trace levels.

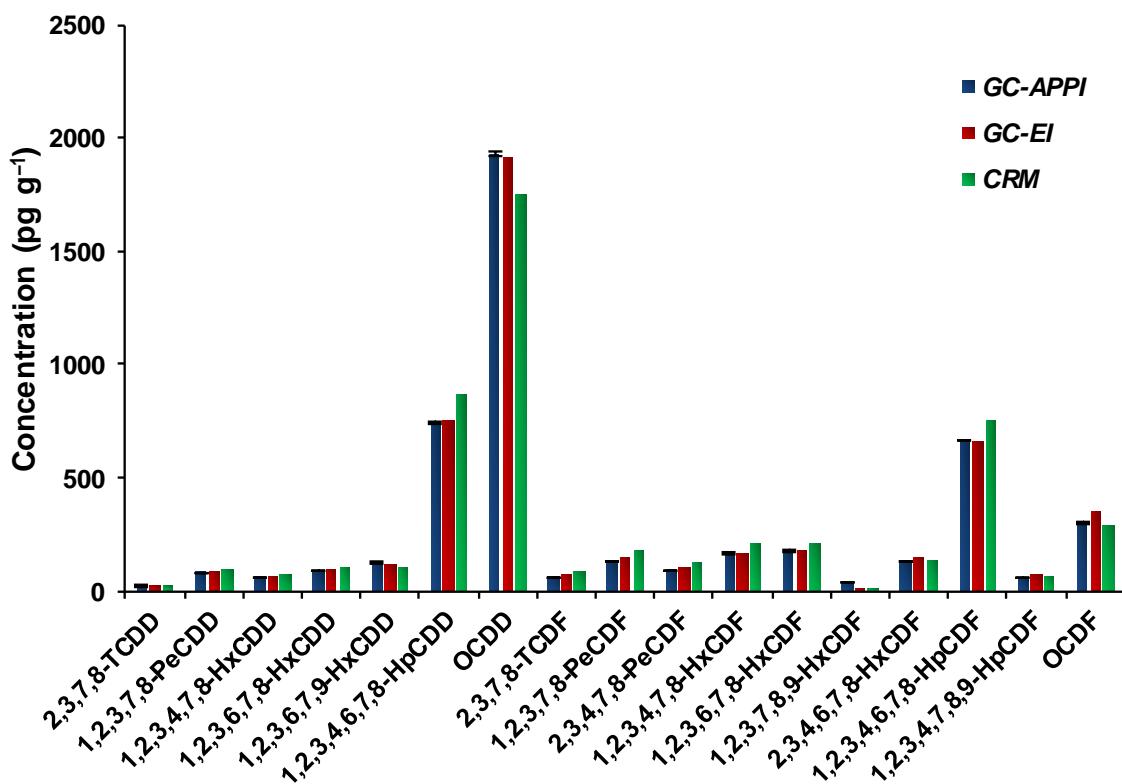


Figure 3. Concentration profile for PCDDs and PCDFs in a fly ash certified reference material obtained by GC-APPI-HRMS and GC-EI-HRMS.

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