DETERMINATION OF HIDDEN ORGANOCHLORINATED CONTAMINANTS IN ENVIRONMENTAL SAMPLES BY GC-MS/MS

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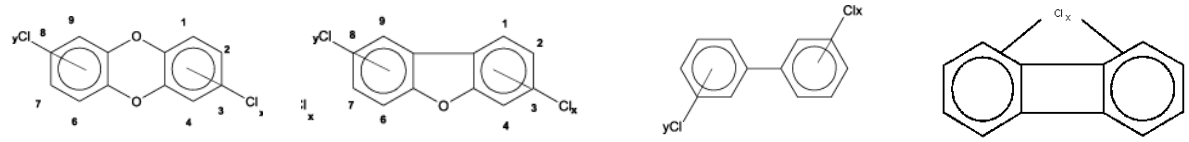
Introduction

A wide variety of chlorinated aromatic compounds are released intentionally and/or unintentionally in the environment. Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and polychlorobiphenyls (PCBs) are well known environmental contaminants. Another class of organochlorinated compounds consists of polychlorinated biphenylenes (PCBPs). They are tricyclic compounds resembling closely PCDDs and PCDFs (figure 1).



Polychlorinated dibenzo-p-dioxins (PCDDs) | Polychlorinated dibenzo-furans (PCDFs) | polychlorobiphenyls (PCBs) | **polychlorinated biphenylenes (PCBPs)**

Figure 1 – General molecular structure of PCDDs, PCDFs, PCBs and PCBPs

Although polychlorinated biphenylenes do not belong to the most often-mentioned pollutants, toxicity of biphenylene and its chlorinated derivates was reported[1,2]. The 2,3,6,7-TetraCBP (2367-TeCBP) isomer was found to be equipotent with 2,3,7,8-TCDD in inducing microsomal enzymes[3], one measure of toxicity in animals. Moreover, studies by Poland and coworkers showed that 2367-TeCBP exhibited AhR-dependent potencies similar to that of TCDD[5].

Despite these assumption, there are very limited and dated studies on this toxic chemicals except observations on building fires[2,6,7].

This work describes the simultaneous determination of 2367-TeCBP in flue gas emission samples by gas chromatography coupled to triple quadrupole mass spectrometry.

Materials and methods

Sample collection and extraction

Flue gas emissions were collected with a stack gas sampler on the filter/condenser method following European reference methods[8,9] requirements. Once collected, the samples were sent to the laboratory for the extraction. Each sample was spiked with standard solutions containing dl-PCBs and PCDD/F (WP-LCS and EPA 1613-LCS, Wellington Labs), prior to the extraction process (36h Soxhlet extraction with toluene).

Sample clean-up

The purification and fractionation of each sample was achieved by an automated system (PrepLinc™ System, J2 Scientific), based on gel-permeation chromatography and alumina SPE cartridge with in-line evaporation module[10].

Instrumental analysis

The gas chromatography/mass spectrometry (GC/MS) analyses were run on a Trace GC Ultra gas chromatograph equipped with a TriPlus autosampler and coupled by a heated transfer line to a TSQ Quantum Triple Quadrupole GC-MS/MS spectrometer (Thermo Scientific). Chromatographic separations were performed using a VF-Xms fused silica capillary column (60 m x 0.25 mm i.d 0.25 µm., film thickness; Varian, Inc.). The experimental conditions were as follows: helium carrier gas (1 ml/min); split/splitless injection of 2 µl. The following temperature program was used: isothermal at 150°C for 1.60 min, 8°/min to 250°C (hold for 10 min) and 8°/min to 325°C, isothermal for 10 min. MS operating conditions were the following: positive electron ionization (EI+) mode with electron energy of 70 eV and an emission current of 50 µA. The transfer line and ion source temperatures were kept at 280 and 250°C, respectively.

Results and discussion

GC/MS optimization

The first step of the method development procedure was to establish the chromatographic retention time of each analyte of interest and to select an appropriate precursor ion for each analyte. This was carried out using full scan GC-MS analysis of a mixture of ^{13}C-PCBs (PCB-LCS-H, Wellington Labs) and ^{13}C-2367-tetrachloro-biphenylene (MCBP-2367, Wellington Labs). More peaks are visible with *m/z* ratios larger than the molecular ion peak due to isotope distributions. Table 1 lists the peaks, the isotope cluster and the theoretical abundance of ^{13}C-TeCB and ^{13}C-TeCBP, respectively. The three most abundant precursor ions produced by electron ionization (EI) from each congener's full scan spectrum were selected as the precursor ions for the sequential application of MS/MS, considering the loss of 2 atoms of chlorine ($^{35}Cl_2$ = -70 *m/z*) by applying an optimized collision energy of 28eV.

m/z	**299.9 (a)**	**301.9 (b)**	**303.9 (c)**	**305.9**	**307.9**
^{13}C-TeCB		M (77%)	**M+2 (100%)**	M+4 (49%)	M+6 (11%)
^{13}C -TeCBP	M (77%)	**M+2 (100%)**	M+4 (49%)	M+6 (11%)	

Table 1 – Isotopic pattern and relative abundances of precursor ion of the mass labeled TeCB and TeCBP. (a), (b) and (c) refers to the transitions considered (see chromatograms in figure 3)

Among the PCBPs, this work was focused only on the determination of 2367-TeCBP, considering its potential toxicity and the availability of the labeled standard. The labeled standard PCB-LCS-H used in this study was chosen because it comprises almost all the PCBs listed in EPA 1668B and UNI EN-1948 methods.

Wakimoto *et al*. observed that several chlorinated compounds, however, interfere in the determination of 2367-TCBP and, though some of them can be avoided using gas chromatography and high resolution mass spectrometry, interference by some compounds could be extremely difficult to overcome[11]. Among the interferents, for example, TeCB [M] and HxCB [M-2^{35}Cl] have the same molecular weight as that of [M+2] and [M] of 2367-TeCBP, respectively. We can assume that HxCB [M-2^{35}Cl] is present in the MS analyzer because of the EI in the MS source. SIM chromatograms of the labeled standards (figure 2) show the chromatographic separation of these compounds. PCB-LCS-H lacks of 60L (sampling standard for EN-1948 method); the sampling standard was analyzed separately, with the same GC conditions used, and the peak (retention time: 19.53 min) does not interfere with 2367-TeCBP.

High certainty of compound identification is always the primary concern in the analysis of environmental samples for trace levels of toxic compounds. One of the most important criteria for identifying trace organochlorinated compounds in a sample, is that the observed isotope ratio of two ions monitored for each isomer, must agree with the Theoretical Isotope Ratio (TIR) (see table 1) within a certain tolerance limit, e.g., ±15% for tetrachloro isomers[9,12].

As tetrachlorinated biphenylenes consists of 22 congeners, in the environment they could be potentially found as a mixture with PCBs. Among these congeners, this work focalized on the determination of 2367-TeCB, as it is the only standard commercially available, but some of them could overlap with dioxin-like PCBs.

If a chromatographic separation between TeCBP and TeCB could not be achieved, the theoretical mass-spectrometric resolving power (R) needed to separate the compounds, considering M (TeCB) = 289.92232 and M+2 (TeCBP) = 289.90373, is R>15000.

It means that a resolving power of 10000, required for PCB analysis by European reference EN-1948[9], is not sufficient.

Since chlorine has two natural isotopes, ^{35}Cl and ^{37}Cl, with an abundance ratio of 3:1, compounds containing different numbers of Cl should give different chlorine patterns by mass spectrometry. But if the concentrations of this compound were too low, or the chlorine pattern of this compound was partly overlapped with those of other compounds in a mixture, it may be difficult to detect it.

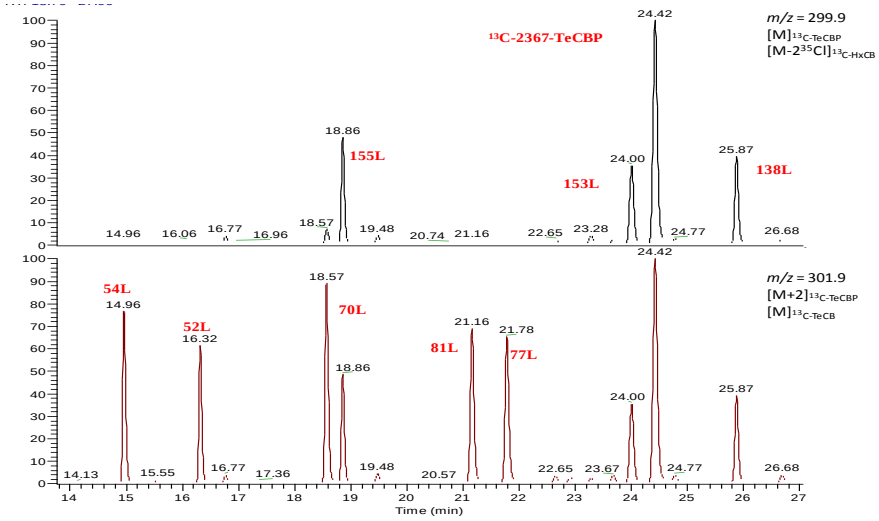
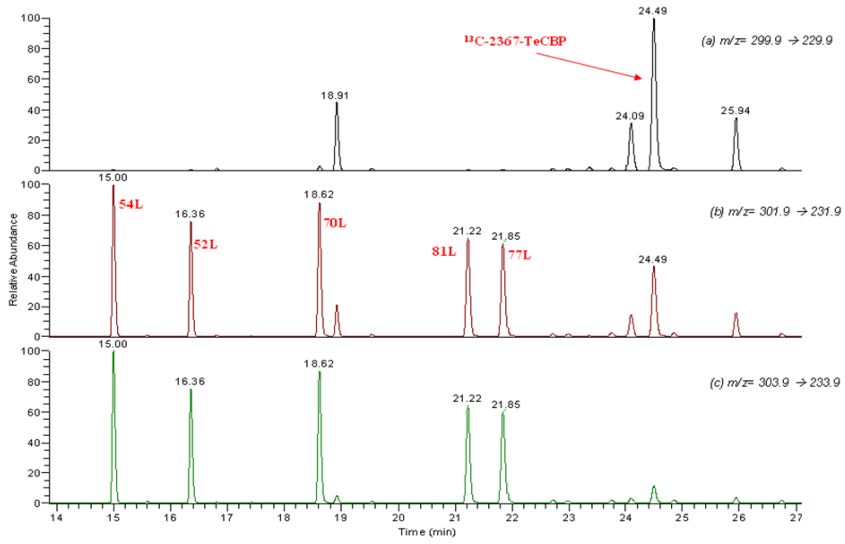


Figure 2 – SIM chromatograms of PCB-LCS-H and MCBP-2367.

Even with the application of MS/MS it is not possible to discriminate HxCB [M-2^{35}Cl] from TeCBP, while it is possible for TeCB from TeCBP (figure 3): the ratios between the areas of the peaks originated from the common transitions (a) and (b) are the same for both HxCB [M-2^{35}Cl] and TeCBP, while they are different between TeCB and TeCBP , as shown in the table.



	$\frac{Area(a)}{Area(b)}$	$\frac{Area(b)}{Area(c)}$
^{13}C-TeCBP	1.6	6.1
^{13}C-HxCB [M-2^{35}Cl]	1.6	6.1
^{13}C-TeCB	0.008	1.5

** mean values of 5 TeCB isomer area*

Figure 3 – MS/MS chromatograms of PCB-LCS-H and MCBP-2367 and relative peak area ratios. Peaks at 18.91, 24.09 and 25.94 min refers to [M-2^{35}Cl] of 155L, 153L and 138L HexaCBs.

Clean-up

Standard stock solutions and flue emission samples were processed with the automated clean-up system described in the method section. Briefly, the sample in dichloromethane was loaded on the gel permeation column, and then the collected fraction, once concentrated by the inline evaporation module, was loaded on the alumina SPE cartridge[10]. Automatically, the system collects in two separate fractions PCDD/Fs and PCBs. Since PCBPs have intermediate properties among PCBs and PCDD/Fs, the optimization of their separation and purification is still ongoing.

Real samples

Flue gas emission samples were analyzed with the same GC/MS conditions in a qualitative aspect. Figure 4 shows a full scan chromatogram and the spectrum related to the peak at 22.73 min. We assume that it is TeCBP, having same retention time and spectrum of the standard.

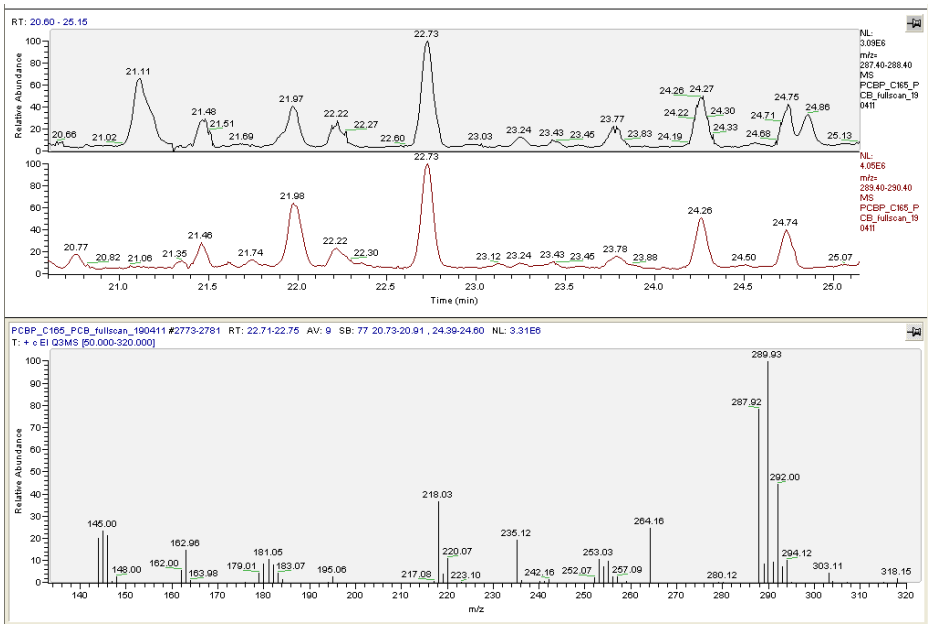


Figure 4 – Full scan chromatogram of a flue gas stack emission sample. The spectrum, referred to RT 22.73, shows the presence of a TeCBP.

Conclusions

TeCBPs are present in flue gas stack emission samples and, because of their potential toxicity, they should be added in the environmental analysis and included in the complete dioxin TEQ value, first having assigned an appropriate TEF, as dl-PCB. Particular attention must be kept in their determination and quantification because they could be overvalued for the overlapping of other chlorinated compounds. In addition, their presence in the samples could interfere with the quantification of dl-PCBs or PCDD/F with the same molecular mass.

References:

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