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APPLICATION OF ION TRAP TANDEM MASS SPECTROMETRY TO THE ANALYSIS OF DIOXIN-LIKE PCBS IN ENVIRONMENTAL SAMPLES

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Introduction

Polychlorinated Biphenyls (PCBs) are being the subject of a broad spectrum of analytical and environmental studies due to their ubiquitous properties, environmental persistence and high potential for bioaccumulation through the food chain. The most toxic congeners are those adopting a coplanar configuration similar to that of the 2,3,7,8- tetrachlorodibenzo-p-dioxin (2378-TCDD), and therefore show toxicological properties resembling those of the 2378-TCDD. They are referred as "dioxin-like PCBs", namely those with no chlorine atoms in *ortho*- positions (*coplanar*-PCBs, IUPAC Nos. 77, 81, 126 and 169) or with only one chlorine atom in one of the four *ortho*-positions (*mono-ortho*-PCBs, IUPAC Nos. 105, 114, 118, 123, 156, 157, 167 and 189)[1]. Some authors have also identified *di-ortho*-congeners (IUPAC Nos. 170 and 180). All of them have been attributed a Toxic Equivalent Factor and should be considered when estimating total dioxin toxic content of environmental and biological matrices as well as in feedings and foodstuff samples. However, levels of dioxin-like PCBs are much lower than those with non-planar configuration and therefore extensive clean-up steps are involved to eliminate the latter. Presently, PCBs determination is based on US EPA Method 1668, wherein High Resolution Mass Spectrometry is applied[2]. Nevertheless, the use of HRMS is considered too expensive and with high cost of maintenance. Alternative methods of lower cost and more accessible to research laboratories would be desired, allowing provision of reliable results about the presence of these compounds in the environment.

The aim of this work is the optimisation of parameters for a quadrupole ion-trap mass spectrometer working in tandem, to be applied to the analysis of dioxin-like PCBs as alternative method to high resolution mass spectrometry. The method is based in sequential sections, wherein different RF potentials and ramping voltages are applied to the ion-trap electrodes in order to achieve ion formation, isolation, collision-induced dissociation (CID with helium) and mass scanning of 14 dioxin-like PCBs (*mono-ortho*, *di-ortho* and *non-ortho* PCBs) and 17 $^{13}C_{12}$-dioxin-like PCBs (isotopic dilution method). Dioxin-like PCBs in sewage sludges and certified reference solutions from two intercalibration exercises have been analysed. Previous specific clean-up steps were needed to separate each fraction from bulk PCBs and other molecules. The comparison of results obtained with those from intercalibration exercises showed the viability of this technique as alternative to HRMS and pointed its large possibilities for the analysis of persistent halogenated micropollutants**.**

Methods and Materials

Chemicals and reagents

All chemicals employed during extraction and fractionation stages were of high purity for pesticide residue analysis. Solid samples were spiked prior extraction with a known volume of Labelled Compound Stock Solution EPA-1668LCS containing following isomers of PCBs: one tetra-chlorinated ($^{13}C_{12}$-TeCB 77L), three penta-chlorinated ($^{13}C_{12}$-PeCB 105L, $^{13}C_{12}$-PeCB 118L y $^{13}C_{12}$-PeCB 162L), four hexa-chlorinated ($^{13}C_{12}$-HxCB 156L, $^{13}C_{12}$-HxCB 157L,

$^{13}C_{12}$-HxCB 167L y $^{13}C_{12}$-HxCB 169L), two heptachlorinated ($^{13}C_{12}$-HpCB 180L y $^{13}C_{12}$-HpCB 189L) and one deca-chlorinated ($^{13}C_{12}$-DCB 209L), at concentration levels from 1000 to 2000 pg μL^{-1} in nonane. In order to calculate analytical recoveries, purified extracts were spiked prior injection with a known volume of Internal Standard Stock Solution EPA-1668ISS containing $^{13}C_{12}$-TeCB 52L, $^{13}C_{12}$-PeCB 101L, $^{13}C_{12}$-HxCB 138L y $^{13}C_{12}$-HpCB 178L at 1000 pg μL^{-1} in nonane. A five-point Calibration and Verification Solution EPA-1668CVS was also used to obtain calibration lines for the native-PCBs congeners of interest, as well as to calculate RFs (response factors, labelled to internal standard) and RRs values (relative response, labelled to native). All solutions were supplied by Wellington Laboratories, Ontario, Canada.

Samples

In order to assess suitability of the method for qualitative and quantitative determination of PCBs, toxicity of two groups of certified reference materials was evaluated. First group comprised two liquid samples (A, B) from 6th Round Intercalibration Study (2001), whilst second contained three solid samples of sediments (C, D, E) from 7th Round Intercalibration Study (2002), both organized by Professor Van Bavel, Orebro University, Sweden. Liquid samples were evaluated in triplicate and solid in duplicate, according to Method US EPA-1668 for the analysis of toxic polychlorinated biphenyls by isotopic dilution.

Sample extraction and purification

Liquid samples were spiked with known volumes of solutions EPA-1668LCS and EPA-1668ISS and then injected directly, undergoing no stage of sample extraction or purification.

Similarly, solid samples were spiked with a known volume of solution EPA-1668LCS and Soxhlet-extracted with toluene for 24 hours. Extracts obtained were later concentrated in rotavapor and solvent was exchanged to n-hexane prior purification Clean-up treatment was carried out in an automated Power Prep™ System (FMS, Inc., USA), wherein sample was forced to pass consecutively and under pressure, through three chromatographic columns packed with different adsorbent materials: multi-layered acid/basic silica gel, alumina and active-carbon PX21[3]. Once sample was run into the system, columns were first eluted with n-hexane in order to drive compounds from silica to alumina column. Next, by eluting alumina with a mixture of hexane:dichloromethane 98:2, some of the *mono-ortho*-PCBs (Fraction I) were recovered. A subsequent elution of hexane:dichloromethane 50:50 through the alumina and active-carbon columns allowed collection of remaining *mono-ortho*-PCBs (Fraction II) and to migration of *non-ortho*-PCBs from the former column to the latter. These were extracted from carbon column by eluting with toluene in counter-current (Fraction III). Fractions I and II were put together and analysed for *mono-ortho*-PCBs. Likewise, Fraction III was first concentrated and then analysed for *non-ortho*-PCBs.

Instrumental

Analysis were made in a VARIAN SATURN 2000 GC/MS/MS (Ion-Trap), equipped with gas chromatograph CP-3800 and programmable injector 1079 (Varian, Walnut Creck, CA, USA). Software version 5.51 was employed. Samples were splitless-injected by means of an autosampler CP-8200 in a fused-silica capillary column BD-5ms (30 m., 0,25 mm. of ID., 0,25 µm film thickness) (J&W Scientific, CA, USA). Chromatographic conditions were as follows: Injector: 70º C, then 300º C (hold 30 min.) at 200º C/min.; Splitter: initially On, then Off for 0,2 min. and finally On again; Splitter flow: 60 ml/min. Samples were injected in nonane (sandwich injection) at a rate of 0,5 µL/s., with volumes varying from 1 to 3 µL. Column oven: 60º C (hold 3 min.), 60-235º C (hold 10 min.) at 25º C/min., 235-275º C (hold 3 min.) at 10º C/min., and finally 275-350º C (hold 3 min.) at 10º C/min. Helium was used as carrying gas at constant flow of 1 ml/min. Temperature of the transfer line was set at 280º C and the corresponding in the trap at 250º C.

Results and discussion

Once the elution order was known (from literature) and retention time fixed for each of the 31 congeners of PCBs (17 compounds of $^{13}C_{12}$-PCBs and 13 of ^{12}C-PCBs), optimisation of MS/MS conditions was attempted[4]. At a very first stage, standard solution EPA-1668CVS was analysed in scan mode (electron impact, 70 eV) for setting most abundant molecular ions for each congener. From tetra- to hexachlorinated isomers M+2 was found to be predominant in the cluster, M+4 for heptachlorinated, and M+6 for the decachlorinated. Isolation of referred molecular ions within the trap cavity for latter fragmentation was accomplished through an Axial Modulation Process, based on applying different radiofrequency potentials to the ion trap electrodes. Considering a q_z value of 0,4 (dimensionless parameter controlling trajectory of ions in the quadrupole field of the ion-trap cavity), software toolkit was used to optimise Excitation Storage Level (ESL). This magnitude, specific for each ion to be isolated, allows both selective ejection of unwanted and confinement of desired molecular ions in the ion-trap (see Table 1).

Table 1: MS/MS parameters for the analysis of toxic polychlorinated biphenyls

COMPOUND	Molecular Ion (m/z)	Product Ion (m/z)	CID (v)	Excitation Time (ms)	Excitation Storage Level (mz)
TeCB	291,2 [M+2]	220/222	1,80	5	128,8
^{13}C-TeCB	303,96 [M+2]	232/234	1,40	5	134,0
PeCB	325,88 [M+2]	254/256	1,75	20	143,8
^{13}C-PeCB	337,92 [M+2]	266/268	1,95	20	149,0
HxCB	359,84 [M+2]	288/290	1,90	20 [(a)]	158,8
^{13}C-HxCB	371,88 [M+2]	300/302	1,65	20	164,0
HpCB	395,80 [M+2]	324/326	2,55	20 [(a)]	174,8
^{13}C-HpCB	407,84 [M+2]	336/338	3,20	20 [(b)]	180,0
^{13}C-DCB	512 [M+2]	440/442	1,60	20	226,0

(a) 5 ms for HxCB 169 and HpCB 170; (b) 50 ms for ^{13}C-HpCB 178L.

When parameters controlling isolation of molecular ions were optimised, next stage consisted of producing optimum fragmentation of such ions. This occurs by collision induced dissociation (CID) with helium molecules in the ion-trap. Fragmentation process depends on: type of collision (excitation mode), number of collisions (excitation time) and relative energy of collisions (excitation amplitude). In order to increase selectivity of the method, Resonant Excitation Mode was selected. Contrary to Non Resonant Mode in which instant shifts on potential energy of trapped ions is produced, Resonant Mode causes an increase in energy coupled to oscillation movement of confined ions, giving rise to fragmentation. Automated Method Development (AMD) software toolkit allowed simultaneous optimisation of the other two parameters: excitation time values varied from 5 to 50 ms, whereas those corresponding to excitation amplitude from 1,40 to 3,20, depending on the congener (see Table 1). Finally, calibration lines were obtained for all native PCBs congeners in the range 2-2.000 pg/µL, with linear responses in agreement with acceptance criteria set in Method US EPA-1668 (variation coefficient < 15%, n = 15).

Mean target/measured values for PCBs toxic content in samples analysed along with Z-score factors are listed below in Table 2. Referred coefficient is a factor framed within the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories (IUPAC). According this parameter, method performance may be estimated as follows: $|z| \leq 2$, satisfactory; $2 < |z| < 3$, questionable; $|z| > 3$, unsatisfactory. As seen in table, z-score

values below 2 were obtained in all cases, indicating goodness of method optimised for these kind of matrices. However, further research would be desired in order to estimate whether method performance maintains with other matrices.

Table 2: Target and measured PCBs toxic content in samples analysed. Samples A and B are expressed in pg I-TEQ/µl and Samples C, D and E in pg I-TEQ/g.

SAMPLE	Target Value (X_t)	Measured Value (X_m)	Z-score Factor ($Z = (X_t-X_m)/\sigma_t$)
Sample A	4.251	3.93	0.386
Sample B	1.804	1.610	0.514
Sample C	0.0045	0.0057	0.621
Sample D	0.0201	0.0188	0.576
Sample E	0.0052	0.0053	1.199 (*)

(*) Since σ_t (referred to total toxicity of the sample) was not available for all target values from intercalibration exercise, 1,199 corresponds to the average of z-scores calculated for each of the twelve congeners assessed (in pg/g).

References

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