P01: FOOD CONTAMINATION BY ORGANOCHLORINES: ANALYSIS OF OYSTER TISSUE

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Abstract – Organochlorines (OCs) such as OC pesticides and polychlorobiphenyls are widespread toxic contaminants in the food chain, especially in aquatic biota. Despite they were banned by many years in western countries they still represent a topic of concern as addressed in the Stockholm Convention on Persistent Organic Pollutants (POPs) currently in force. The analyses of POPs at trace level in biological matrices may represent a challenge: if not properly conducted they may suffer of great uncertainties. In the present work the international guidelines about Good Laboratory Practice are strictly followed to analyse oysters and mussels. Other analytical innovations, recently developed in our laboratory, were applied in order to further improve accuracy. The oyster tissue is part of the analytical activity for producing a new Reference Material within the H2020 Project "PRO-METROFOOD".

Keywords: Persistent Organic Pollutants (POPs); Organochlorines (OCs); Aquatic biota; Organochlorine pesticides (OCPs); Polychlorobiphenyls (PCBs); Good Laboratory Practice (GLP)

1. INTRODUCTION

Organochlorine pesticides (OCPs) and polychlorobiphenyls (PCBs) are chlorine-derived compounds enormously popular in the past and produced worldwide: the global production of DDT in 1974, for example, was 60,000 tons [1]. After massive use and consequent dispersion in the environment the negative impact on human health of OCs became later evident with a number of symptoms, included carcinogenesis, also at trace levels. Not degradability and high solubility in the organic matter allowed them to enter in the food chain as contaminants therefore they were banned for use in most western countries more than thirty years ago.

Nevertheless today OCs are ubiquitous and still detectable in the entire food chain, especially in seafood [2-3]. This caused the Stockholm Convention on POPs to enter into force on 2004: controlled disposal of organochlorines still available and global ban for use were proposed [4]. To date 180 countries have ratified the Convention.

The high toxicity of these xenobiotics even at trace level makes their analysis a delicate matter. IARC (International Agency for Research on Cancer) has recently classified as carcinogenic to humans the entire PCB group, included the non dioxin-like PCBs (NDL-PCBs) by affirming that the carcinogenicity of PCBs cannot be solely attributed to the dioxin-like congeners [5]. The recent EU regulation fixed a maximum level for the sum of the six indicator NDL-PCBs equal to 75 nanograms per gram of fish fillet, i.e., 75 parts per billion [6]. The need for a laborious purification step together with a perfect instrumental setting made it possible that frequent large errors by non-specialized laboratories are made. As cited in a QUASIMEME performance study in 1994 [7] "The bias obtained by the participating laboratories for the determination of PCBs and OCPs is currently too large to meet the requirements of international monitoring programmes".

Our laboratory specializes in OCs analysis and in the present work the international recommended guidelines about GLP are strictly applied [8]. Moreover some innovative techniques recently introduced by us in the field of Mass Spectrometry [9] made it possible that some known analytical problems were addressed, so further improving accuracy.

2. EXPERIMENTAL

2.1 Materials and Methods

2.1.1 Reagents and Chemical Standards

All reagents were of pesticide grade. Acetonitrile, acetone, isooctane, n-hexane,

petroleum ether 40–60 °C, toluene, methyl alcohol, dichloromethane, ethyl acetate, sodium sulphate and Florisil® 60–100 mesh were purchased from Carlo Erba Reagents®. The Supelclean LC-18 solid phase was from Supelco® (Bellefonte, PA, USA). Pure standards of OCPs and PCBs were purchased from different producers, mainly in solution form, along with their certificates of analysis. Certified solutions, as single standard or mixtures, were from Dr. Ehrenstorfer® (Augsburg, Germany), AccuStandard® Inc. (New Haven, CT, USA), Supelco® (Bellefonte, PA, USA) and Riedel-de Haen/Fluka/SIGMA ALDRICH® (Switzerland).

2.1.2 Oyster and Mussel Samples

The Oyster sample to be characterized within the H2020 project "PRO-METROFOOD" was delivered in lyophilized form: five aliquots of 25 g in plastic bottles (RM 003, n. 36 to 40).

The certified reference material "lyophilized Sea Water Mussel SQC068MUS-30G, lot LRAA9269" was from Sigma-Aldrich®.

2.1.3 Sample Preparation

About 2.5 g of lyophilized sample were exactly weighed. Samples were subjected to multiple purification steps by using the solid phase extraction technique. In the first purification diatomaceous earth and C18 were used while in the second purification the solid phase was constituted by Florisil®. The procedure was already described in any detail [9-10].

2.1.4 Gas chromatographic analysis

Two identical capillary columns (RTx®-PCB Restek, 60 m × 0.25 mm ID, 0.25 µm df) were mounted on two separate injectors and were installed in the same oven (Varian® 3800 GC). The first column was connected to an electron capture detector (ECD) while the second was connected to the Mass Spectrometer Varian® Saturn 2000 that is Ion Trap - equipped. Instrumental conditions were as already reported [9]. In each analysis the sample was first injected in the ECD-column and then in the MS-column. Calibrations were performed by using standard solutions of the OCs under study that were injected on both columns at appropriate concentrations (multi-level calibration).

2.2 Analytical Quality Control

According to the recommended guidelines the following checks are regularly performed in the laboratory: linearity of instrumental response, limit of detection (LOD), limit of quantitation (LOQ), analysis of certified reference materials, recovery assays, blanks, interlaboratory ring tests [9-11].

3. RESULTS AND DISCUSSION

The characterization of the oyster tissue within the PRO-METROFOOD project is currently ongoing and the definitive results will be available only when the work will be completed. However a Certified Reference Material (CRM) very similar to the oyster tissue under study was purchased and analyzed in order to ensure that good quality results are released by the laboratory. Such CRM (lyophilized mussel tissue) was processed in the same exact way as done for the oyster sample and it is therefore very suitable to describe the quality controls and the innovations applied in the present study.

Table 1. Analysis of the certified reference material "Sea Water Mussel" (µg/kg).
N. of analyses = 2

PCB	measured values		certificate	
	GC-ECD	GC-MS	certified value	acceptance interval
28	138 ± 3	174 ± 20	212	117 - 307
52	104 ± 3	125 ± 10	120	66 - 174
101	181 ± 4	213 ± 17	226	124 - 328
118	133 ± 2	142 ± 0	115	63 - 167
114		148 ± 22	131	72 - 190
138		178 ± 14	213	117 - 309
153		267 ± 19	309	170 - 448
157		215 ± 23	198	109 - 287
180		234 ± 11	233	128 - 338

Table 1 reports the performance achieved with the cited CRM: the optimum agreement between the measured values and the certified ones should be noted. The recovery of the Internal Standards added to the sample (surrogates) was constantly 100% (PCB 5 = 116.5 ± 0.1 %, 2,2'-DDE = 102.2 ± 1.5 %, PCB 198 = 100.8 ± 3.0 %) and therefore no corrections for recoveries were needed. The same was observed for the oyster tissue.

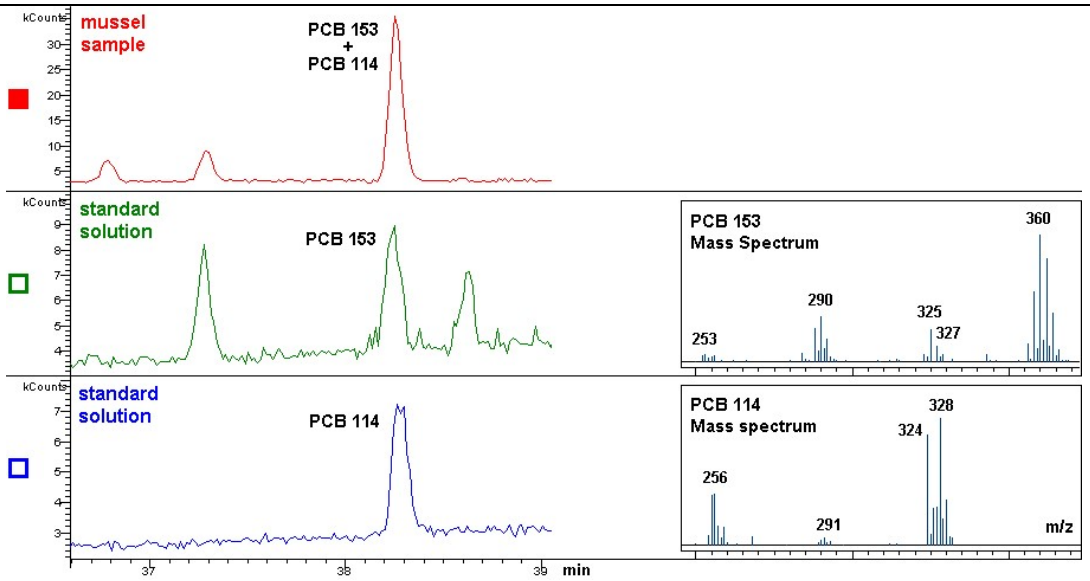

Figure 1. On the left: GC-MS chromatograms. From top to bottom: mussel sample, standard solution of PCB 153, standard solution of PCB 114. Full Scan mode. On the right: Mass spectra of PCB 153 and PCB 114.

The instrumental procedure applied in our laboratory deal with a double GC – double detector technique: results coming from GC-ECD are compared with the results coming from GC-MS. Especially PCBs represent a well-known analytical challenge. This is caused by the fact that, at now, a single GC column that is able to separate all the 209 existing congeners does not exist.

In a recent research [9] we introduced innovations to effectively resolve some of these old problems related to PCB analysis. One important innovation was a new treatment of the *coelutions of contiguous homologs*, i.e. the coelutions between a superior congener with an inferior one (hexa-Cl and penta-Cl for example). The innovation was successfully applied in the present work, as exposed below.

Fig. 1 shows what we observed in the Mussel sample: the peak around 38.3 min is the sum of PCB 153 and 114. Therefore we are facing with a coelution between a superior and an inferior congener. The ECD detector is useless in these cases. It is well known, instead, that Mass Spectrometry can quantify the superior congener (PCB 153) also in the presence of the coelution, by selecting its molecular ion 360 m/z (SIM mode or extracted ion mode). In fact, in the mass spectrum of PCB 114 it is not present the ion 360 (Fig. 1).

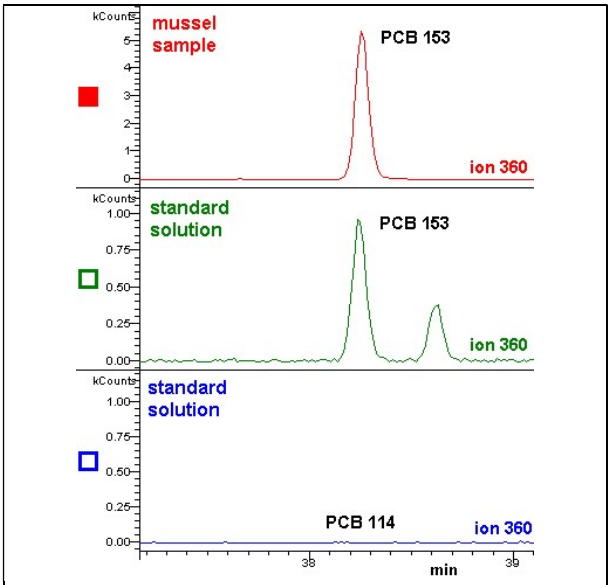

Figure 2. GC-MS chromatograms of Fig. 1. Extracted Ion mode: ion 360.

By integrating the ion 360 in the Mussel sample the contribution of PCB 114 will be zero and only PCB 153 will be correctly quantified as Fig. 2 clearly shows.

Until now it was commonly believed that an accurate quantification of the inferior congener (PCB 114 in this case) was not possible even by using Mass Spectrometry [12]: it was mistakenly thought that the dechlorination ionic cluster around 325-327 m/z present in PCB 153 interferes with the

molecular ionic cluster of PCB 114 around 324-328 m/z (Fig. 1).

The new treatment of the coelutions of contiguous homologs firstly introduced by us in a 2015 research [9] has corrected this erroneous belief. We highlighted that all PCBs have an alternation of even-odd ion clusters, as Fig. 1 shows. The first dechlorination cluster 325-327 m/z of PCB 153 does not contain even ions in an appreciable way, while the molecular ion cluster of PCB 114 does not contain odd ions in an appreciable way. We experimentally demonstrated that by selecting the molecular ion of the inferior congener (328 m/z for PCB 114) quantification can be achieved with no errors. Fig. 3 provides a clear confirmation in this regard. This procedure is valid for all possible coelutions of contiguous homologs.

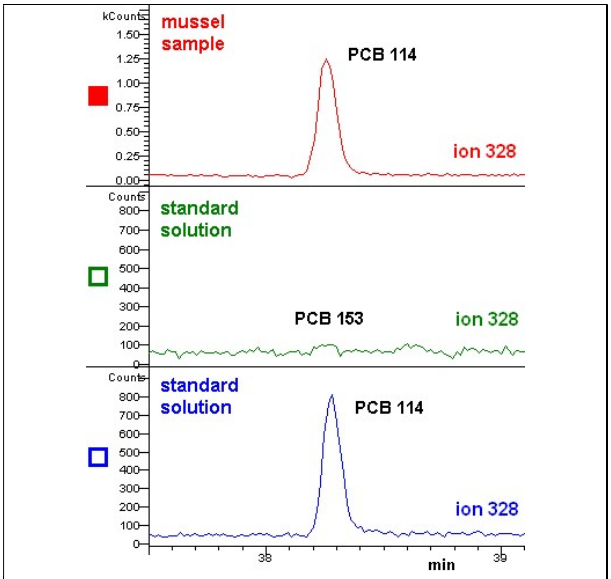

Figure 3. GC-MS chromatograms of Fig. 1. Extracted Ion mode: ion 328.

By integrating the molecular ion 328 m/z of PCB 114 the contribution of PCB 153 is zero. The good accuracy of the procedure was confirmed by comparing the measured values and the certified ones for PCB 114 and PCB 153 in the CRM. The same approach was applied for another coelution of contiguous homologs observed in the mussel sample: PCB 157 and PCB 180.

4. CONCLUSIONS

As expected the POPs characterization of oyster and mussel samples needs quality controls and Good Laboratory Practice at the "state-of-the-art" level.

The challenging task of determining the organochlorine contaminants has requested also the use of an innovative approach to face coelutions between a superior PCB congener with an inferior one.

Being the work still in progress it is to be verified if the cited approach and other innovative techniques recently published (such as the "mass spectrometric ortho effect" [9]) are to be applied to correctly assess the OC content in the oyster tissue.

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