TOWARDS LESS MANIPULATED REFERENCE MATERIALS FOR FOOD AND ENVIRONMENTAL ANALYSIS

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Introduction

The need for reliable and comparable analytical data in food and environmental monitoring stipulate the need for "new/alternative" types of reference materials matching more closely real world samples. Matrix certified reference materials are required in the validation of methods of analyses of real environmental samples, because matrix effects may result in bias of the analytical results. The study was designed to assess the equivalence between three different processing options for the production of food/environmental reference materials.

Finding an appropriate analyte/matrix combination was the first step. A stable analyte, PCBs (polychlorinated biphenyls) was chosen in this case, in order not to complicate the study too much by choosing analytes that could potentially degrade [1]. A fatty fish such as mackerel was chosen as in such matrices the naturally occurring levels of PCBs are sufficiently high not to create too many difficulties for the quantification.

Spiking should be avoided because this would compromise the closeness to a real sample and would complicate the homogeneity of the fresh material. The fatty matrix is also a challenge in the processing, as a homogeneous distribution of the fatty parts has to be ensured. PCBs 28, 52, 101, 118, 138, 153 and 180 have been selected because they are frequently determined. They show a high occurrence in environmental and waste samples and represent both lower and higher chlorinated congeners.

This paper summarises the protocol used to determine the homogeneity of the three material presentations. And the results from the analyses carried out to measure the PCB content of batches stored at different conditions. Evaluation of the suitability of the test materials will be performed with respect to its behaviour under different storage conditions.

Materials and Methods

Preparation of the test batch: In this case the raw material was a natural sample and no spiking with PCBs was performed. The material was ordered from a local fishmonger. The material was delivered on ice and immediately subjected to further treatment. It was cut and minced. The initial material was splitted into three parts and each of the materials underwent different treatment (see Figure 1).

Standards and reagents: Solvents used were all of HPLC grade, sodium sulfate was of analytical grade. All solvents and reagents were obtained from Merck KGaA (Darmstadt, Germany).

Crystalline PCB congeners were certified reference materials from IRMM: PCB 28 (BCR-291), 52 (BCR-293), 101 (BCR 294), 118, BCR (295), 138 (BCR-296), 153 (BCR-297), 180 (BCR-298). ^{13}C - labelled PCB congeners were obtained as individual solutions in isooctane from Wellington Laboratories (CAMPRO Scientific, Netherlands).

A stock solution containing 7 PCB congeners was prepared and an aliquot from the stock solution was further diluted for the preparation of calibration standards.

Analytical procedure: Extraction was performed using an accelerated solvent extractor ASE 200 (Dionex, Belgium) equipped with 33 mL stainless steel cells. Approximately 10.0 g of autoclaved and frozen materials

were mixed with 6 g Na_2SO_4 (in order to dry the sample) or 1.0 g freeze dried material. The samples were then extracted with dichloromethane. Prior to extraction, the samples were spiked with the internal standard (the mix of isotopically labelled congeners). The ASE conditions were as follows: temperature = 100 °C, pressure = 138 bar, pre-heating time = 5 min, static time = 3 min, static cycles = 2.

The extracts were evaporated on a rotary evaporator (Laborota 4001, Heidolph Instruments, Germany) to ~ 2.5 mL. The clean-up was done in two steps: first the samples were cleaned-up using Gel Permeation Chromatography (GPC). A GPC system (Accuprep MPS, TM Hochdruck clean-up system) was used. An express column filled with 40 g Envirobeads SX3 (length 750 mm, diameter 20 mm) and dichloromethane as a solvent with a flow rate of 4 mL/min was used. The fraction containing PCBs between 16-22 min was collected.

The second clean-up was performed using Solid Phase Extraction (SPE) cartridges – Bond Elute PCB. For conditioning of the cartridge 1 mL Hexane was used. Then ~ 0.5 mL sample solution was added to the extraction column. The PCB fraction was eluted slowly without vacuum with 2.5 mL Hexane. After the clean-up, extracts were concentrated down to 200 µL by evaporating the remaining solvent.

GC-MS parameters: Analyses were carried out with a GC-MS system from Agilent, Belgium (GC model 6890 coupled to a mass selective detector MSD 5973 inert, operated in the electron ionisation mode; samples were injected by an autosampler model 7683b). The column used was a HT-8, 50 m x 0.22 mm ID and 0.25 µm film thickness. The carrier gas was helium at a constant flow rate of 1 mL/min. The injector was operated in the pulsed splitless mode at a temperature of 300 ºC (injection pulse pressure 45.0 psi). The GC oven was programmed as follows: initial temperature of 90 ºC for 1.2 min; temperature increase at a rate of 25 ºC/min up to 200 ºC; hold at 200 ºC for 10 min; temperature increase with 10 ºC/min up to 260 ºC; hold at 260 ºC for 10 min; temperature increase with 10 ºC/min up to 300 ºC; hold at 300 ºC for 10 min. The total run time was 45.6 min.

Quantification of PCBs was carried out using the isotope dilution mass-spectrometry (IDMS) method. The internal standard (IS) consisted of a mixture of labelled PCB congeners. For each compound, integration was performed using the corresponding labelled congener of the compound being quantified. The concentration of the internal standard was kept almost constant for all calibration levels.

Results and Discussion

Suitability of the material regarding PCB levels and profiles:

Since the analysed material was naturally contaminated with incipient analyte, the PCB congener profile was expected to match closely the distribution profile in fish reported by Voorspoels et al. [2]. The congener profile of the PCBs (normalised to PCB 153) found in each of the analysed materials (autoclaved, freeze dried and frozen) is shown in Figure 2. The presented profiles match the distribution of congeners that can be found in real fish samples. Furthermore it can be concluded that the material processing (e.g. autoclavation or freeze drying) does not influence the profile and the levels of contamination. The levels in the material are representative for natural contaminated fish and as such they are suitable for preparation of the reference material matching the real samples.

Suitability of the material regarding homogeneity

Homogeneity was tested using 14 bottles (two sub-samples of each bottle) for each material, selected at approximately regular intervals to cover the whole of the produced batches. The results of these measurements were evaluated using a method described by Linsinger et al. [3]. To check if the obtained data follow a normal distribution, normal probability plots and histograms were visually inspected. The variation between bottles (s_{bb}) and inhomogeneity that could be hidden by the method repeatability (u_{bb}*) are presented in Table 1.

It seems that the homogeneity is worse for the autoclaved and frozen material as compared to the freeze dried one. Achieving homogeneous distribution of the fatty particles in the fresh material as well as in the autoclaved one is the main problem. The freeze dried material was additionally milled, sieved and thus a better particle distribution was achieved, resulting in better homogeneity.

Conclusions

The chosen material is suitable for the performed study with regard to PCB levels and distribution profile. The main problem is to ensure the homogeneity for the autoclaved or fresh material. An improved cutting and mixing procedure needs to be developed. The frozen material matched the real sample most closely, but the problems producing and distributing such a CRM consist mainly in the transport, because this material would need to be shipped at – 20 °C and this would significantly increase the cost of the material. The freeze dried material is the most homogeneous, but does not match the appearance of real samples. Additionally, some degradation of the matrix was observed upon exposure to higher temperatures, which could potentially occur during storage. The autoclaved material is most advantageous as it matches most closely real samples and is easy to be stored and shipped, but it still requires some improvements of the homogenisation process.

References

[1] Minh T.B., Nakata H., Watanabe M., Tanabe S., Miyazaki N., Jefferson T.A. et al. *Arch. Environ. Contam. Toxicol*. 2000; 39: 398-410.
[2] Voorspoels S., Covaci A., Maervoet J., De Meester I., Schepens P. *Mar. Poll. Bull.* 2004; 49: 393-404.
[3] Linsinger T.P.J., Pauwels J., van der Veen A.M.H., Schimmel H.G., Lamberty A. *Accred. Qual. Assur*. 2001; 6: 20-25.

Figure 1: Flow chart of the set up of the feasibility study

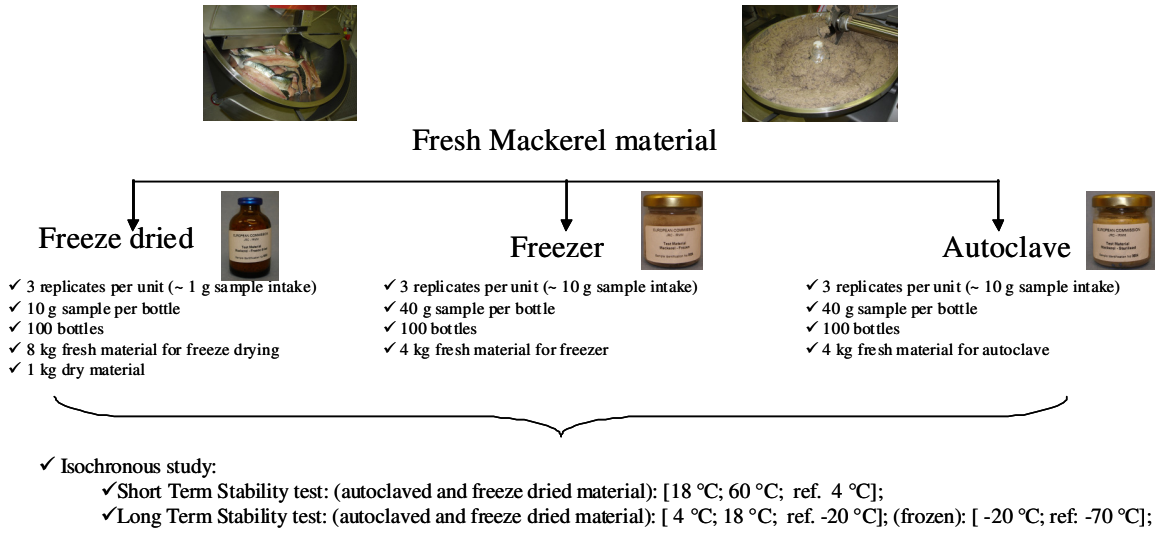

Figure 2: Distribution profile of PCB congeners in autoclaved, freeze dried and frozen material

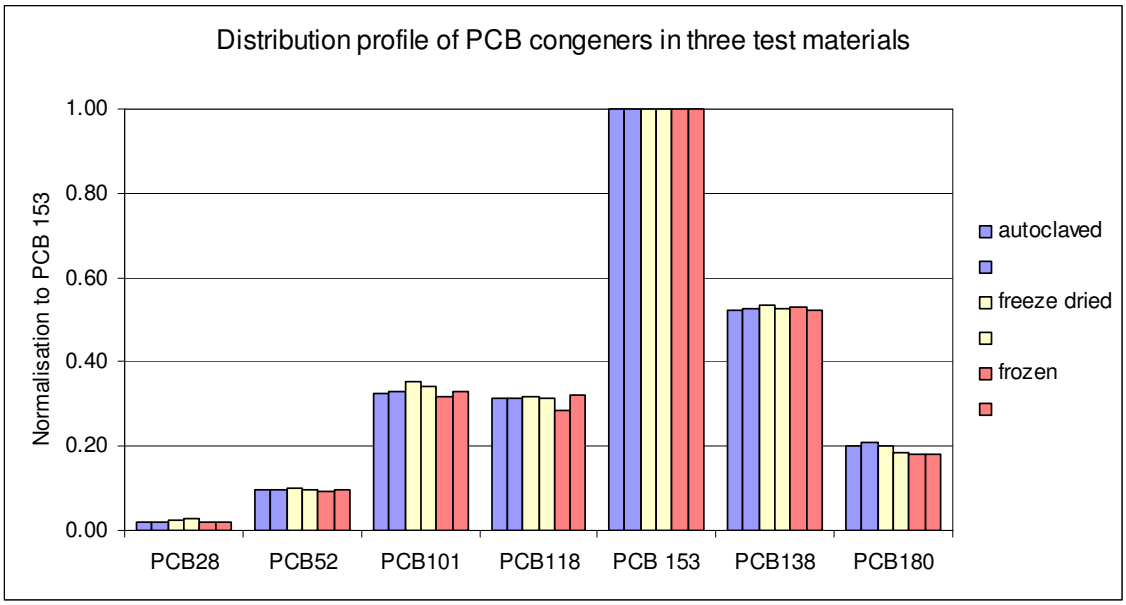

Table 1: Comparison of the results received for homogeneity

PCB #	Autoclaved		Freeze dried		Frozen	
	sbb, %	ubb*, %	sbb, %	ubb*, %	sbb, %	ubb*, %
28	32.0	11.0	14.0	3.0	12.0	17.0
52	14.4	9.0	3.3	1.4	19.7	14.3
101	26.9	10.7	7.5	5.6	17.4	12.1
118	28.0	10.0	1.7	1.9	19.7	15.4
153	28.0	10.1	6.8	7.6	28.0	10.0
138	27.1	10.2	7.8	8.7	18.3	14.7
180	27.4	10.3	1.7	10.7	not defined	20.8