HEXACHLOROBENZENE AND HEXACHLOROBUTADIENE IN FISH TISSUE: METHOD DEVELOPMENT FOR IMPLEMENTATION OF THE WATER FRAMEWORK DIRECTIVE

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Introduction

The production and intensive agricultural or industrial use of persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), have led to the widespread contamination of the environment. POPs are characterised by a high bioaccumulation potential in food chains and therefore may pose a serious threat to upper trophic levels of aquatic communities. In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems[1]. In the frame of the Water Framework Directive (WFD)[2], one of the "daughter" Directive[3] sets environmental quality standards of 10 µg/kg for HCB and 55 µg/kg for HCBD in prey tissue (wet basis) by choosing an appropriate indicator of among fish, molluscs, crustaceans and other biota. The JRC Institute for Reference Materials and Measurements (IRMM) has focused its attention to the provision of a certified reference material for the analysis of hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) in fish tissue. In the process of production of a CRM, a validated analytical method is required for the characterisation analysis of the material. In this study the first steps in the development and validation of a fit-for-purpose analytical procedure for HCB and HCBD in a fish matrix based on GC-IDMS are presented.

Materials and methods

n-Hexane, acetone, dichloromethane, *iso*-octane residue grade, anhydrous Na_2SO_4 and 0.150-0.250 mm florisil for column chromatography were provided by Merck (Darmstadt, Germany). Isotopic labelled (^{13}C) HCB (99.5% w/w) and HCBD (99% w/w) and neat crystals of native HCB and HCBD (certified) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Accelerated solvent extraction system (ASE 200, Dionex) was used in the sample preparation part. Clean-up steps were applied during and after extraction by using a fat retainer and anhydrous Na_2SO_4 exposed to 130 °C for 12 h. Extracts were concentrated and injected into a GC-MS system (Thermo GC 2000-DSQ MS).

Instrumental analysis was carried out by GC-MS in the selective ion monitoring (SIM) mode. A fused silica column, 50 m x 0.22 mm film thickness x 0.25 µm ID HT8 was installed. A programmed temperature vaporizing (PTV) injector was used (temperature programme started at 100 °C and increased to 200 °C by 14.5 °C/min). 1 µL of sample was introduced to the GC/MS. Helium was used as a carrier gas. The ion source and interface temperatures were 250 °C and 280 °C, respectively. The oven temperature programme started at 90 °C (held for 1.2 min), then increased to 215 °C by 25 °C/min (held for 8 min) and finally increased to 300 °C by 25 °C/min (held for 8 min). The quantification was carried out by isotope dilution technique.

Results and discussion:

In the sample preparation part, an accelerated solvent extraction (ASE) method was used to separate HCB and HCBD from fish tissue. The ASE method has many influential parameters like temperature, composition of solvents, static time, cycle number, and flushing volume. The method development has been focused on applying different values of these parameters towards optimised conditions.

The study has been focused on silurus (Silurus glanis). Silurus has a low fat content[4]. It is mentioned in some publications that even as little as 2 % w/w of co-eluted fat could cause a decrease of recovery[5]. Therefore it was decided, as first task, to properly clean-up the sample from the fat. Different fat retainers were used for this

purpose. In the literature it is suggested that the ratio of fat to fat retainer should be maximum 0.0025[6]. Acidic aluminium oxide, florisil and impregnated silica gel (60) were employed as fat retainer following different procedures of activation with regards to applied temperatures and acidic media. Temperature and composition of the solvent mixture used during the extraction are important parameters on co-elution of fat. Several experiments were run in order to find suitable conditions for eliminating the fatty matrix interferences. During method development, the robustness of the extraction step was also tested with different fish matrices having higher fat content compared to silurus like mackerel (approx. 10 % fat content). After establishing appropriate conditions to clean-up the sample from the fat, recovery experiments is ongoing spiking isotopic labelled and native HCB and HCBD standard solutions into the fish matrix.

A method validation plan aiming to assess several parameters (as linearity, limit of detection/limit of quantification (LOD/LOQ), trueness, selectivity, intermediate precision, repeatability and robustness) against pre-defined performance criteria has been established. The validation experiments will be performed by analysing naturally contaminated fish tissue. Trueness will be evaluated by using a certified reference material and by the standard addition method. The validated method will be used in the interlaboratory comparison study for the characterisation of a candidate certified reference material.

The preliminary results acquired in the process of method validation will be shown, together with the achieved validation parameters. An uncertainty budget will be estimated from method validation taking into account contributions from preparation of standards, repeatability, day-to-day variation and trueness.

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