Lipid Reduction during the Analyses of PCDDs, PCDFs and PCBs in Environmental Samples using Semipermeable Membrane Technique.

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During the analysis of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho as well as mono-ortho PCBs in biological samples usually large amounts of lipids have to be removed. Several different techniques have been used in the past as acid or base treatment, gel permeation or other chromatographic columns, supercritical fluid extraction and extraction directly on carbon column. Most of these methods are limited to about 1 gram of lipids due to practical reasons. Often it is necessary to start with large samples (up to 300 gram wet weight) for achieving a good detection limit. Typically the amount of lipid is 1-5 gram in a single sample. In this paper we present a new method for cheaper and rapid lipid reduction, clean up and analysis for PCDDs, PCDFs, non-ortho PCBs and mono-ortho PCBs. The method includes gravity-flow extraction, semipermeable membrane (SPM) lipid reduction[1,2], combined silica column and HPLC carbon fractionation[3]. The final analyses are done with two HRGC/HRMS injections, one for the mono-ortho PCB and one for the other compounds.

Materials and methods

The sample is mixed with approximately 5-20 times by weight dried (530°C, 48h) sodium sulphate in a homogenisator (Tecator 1094 homogenizer, Sweden). A free-floating powder is obtained after 24h. This sample can be subsampled if necessary or, if the whole sample is needed, it can be extracted directly. It is also possible to store the sample in freezer for an extended time at this stage. The extraction is performed in large custom made glass columns (1-1.5 meter high, diam. 4cm) equipped with a removable glass fiber disc (Whatman, GF/D) in the bottom. The internal standard containing 11 different 13C-labelled PCDDs and PCDFs and 5 different 13C-labelled PCBs is added. A common sample size consisting of about

500 gram total sample is extracted with 300 mL acetone/hexane (2.5/1 v/v) and followed with 300 mL hexane/diethyl ether (9/1 v/v). If a larger sample has to be extracted, the solvent volume is increased proportionally. The solvent is collected in a pre weighted and carefully cleaned 1 litre round bottom flask. Before use all glass ware is cleaned three times with each solvent methanol, methylene chloride and toluene. All solvents used in the analyses is of Glass destilled quality (Burdick & Jackson, USA) with the exception of ethanol, tetradecane and diethyl ether.

The solvent is evaporated with a rotavapor (Büchi, RE120, Switzerland). In order to remove the remaining water, 100 mL of 99.5% ethanol is added. The evaporation is carefully done until constant weight is achieved for total lipid determination, which is done gravimetrically. The lipid fraction is redissolved in a total of 20 mL of cyclopentane. The membrane is formed like a tube and heat sealed in one end. This sealed tube is placed into a glass funnel with a Teflon stop cock in the bottom. The SPM is a "layflat" polyethylene tube, 26 mm wide and the membrane thickness is 76,5µm with no additives added during production (Cope plastics, Inc. St Louis, MO, USA). Before use the membrane is cleaned in a beaker with cyclopentane for 24 hours. The extract is transferred to the tube using a Pasteur pipette. Depending of the sample size the glass funnel is between 200-500 mm in height and the internal diameter is 40 mm. The same round bottom flask is placed below the funnel and cyclopentane is added to give the same solvent level as inside the membrane tube giving a volume of approx. 150 mL. The outside solvent is changed after 16 hours, 2 days and 3 days. After 4 days the combined fractions are evaporated down to three mL using a rotavapor. The dialysis reduces the lipid content to about 1-5% depending on dialysis time, lipid nature and solvent system. If it is difficult to dissolve the lipids additional solvent (methylene chloride) could be added for the transfer of the sample giving approx. 5% final methylene chloride concentration in the tube.

Further clean up is made on a combination silica column consisting of from bottom glass wool, cesium silicate, neutral silica gel, 40% sulphuric acid silica gel, neutral silica gel potassium silicate, and dry sodium sulphate (prepared as above). All gels are precleaned with methylene chloride and methanol[4]. These columns have an internal diameter of 20 mm and the length is 320 mm and the elution is by gravity flow. The solvent used is 100 mL 20% methylene chloride in cyclopentane and the eluate is collected in a small conical flask. 80 µL of tetradecane is added and the solvents are removed in the rotavapor. The sample is then transferred to a smaller 10 mL flask and evaporated in the hood to near dryness with only the tetradecane left. Next day the sample is transferred to an autosampler vial (200 µL) with two additional 40 µL portions of tetradecane. For the fractionation of PCDDs, PCDFs,

non-ortho PCBs and mono-ortho PCBs a home made PX21 HPLC column on an HP 1050 HPLC with an autosampler equipped with a Foxy 200 x-y fraction collector (ISCO, USA) were used. Three fractions are collected with a flow rate of 4 mL per minute. In the first fraction one can find most of the common pesticides and 2-4 ortho PCBs. The second fraction contains the mono-ortho PCBs and the third fraction contains the non-ortho PCBs, PCDDs and PCDFs. The fractionation scheme is modified[3] and used as follows: 1% Methylene chloride in n-Hexane for 7.5 minutes, gradient elution up to 10% Toluene for 32.5 minutes and the third fraction is by reverse elution with toluene for 40 minutes. Fraction one is collected during the first 15 minutes and fraction two between 15 and 40 minutes. The reverse elution with toluene gives the third fraction and this is followed by cleaning with Toluene in reverse mode. It is necessary to treat the mono-ortho PCB fraction separately due to the reason that the mono-ortho PCBs are present at concentrations 2-3 orders of magnitude higher than the other compounds of interest. After fractionation the second fraction is evaporated in a conical flask with rotavapor and 40 µL of tetradecane was added as keeper. This sample is transferred to a 10 mL vial with cyclopentane, the recovery standard (100 ng 13C- IUPAC PCB #101; 13C-2,2',4,5,5'-pentachloro biphenyl) is added and then evaporated to near dryness. After dilution to 2mL with cyclopentane, 40 µL is transferred to a new 10 mL vial, 80 µL tetradecane is added and the cyclopentane is evaporated. Then the solution (100 times diluted sample) is transferred to a 200µL autosampler vial ready for HRGC/HRMS analyses. Fraction three is evaporated together with 40 µL tetradecane in a conical flask to near dryness using a rotavapor and the sample is transferred to a 10 mL vial with small portions of cyclopentane. The internal standard (500 pg each 13C-12378-PeCDF and 13C-1234678-HpCDF) is added and after evaporation in the hood the sample is transferred to a 200 µL autosampler vial ready for HRGC/HRMS analysis..

For both fractions a VG 70-250S (Fisons, England) high resolution mass spectrometer running in the EI mode equipped with a 60 m PTE-5 (Supelco Inc. USA) capillary column in the HP 5890A gas chromatograph is used. Selected ion monitoring is used for the molecular ion and one confirmation ion in the chlorine cluster and using 5 different groups during the run of fraction three. PCDDs, PCDFs with 4-8 chlorines and non-ortho PCBs (PCB IUPAC #77, 126, 169; 3,3',4,4'-tetrachloro, 3,3',4,4',5-pentachloro and 3,3',4,4',5,5'-hexachloro biphenyl) are monitored from the same injection. Fraction two is analysed in the same way but with only one group for tetra- to hexa chlorinated biphenyls (PCB IUPAC #105, 118, 156,157; 2,3,3',4,4'- and 2,3',4,4'5-pentachloro biphenyl, 2,3,3',4,4',5- and 2,3,3',4,4',5'-hexachloro biphenyl).

Results and discussion

The use of SPM in the clean up replaces other more costly or laborious steps and also gives the opportunity to use larger sample sizes. One tube easily remove lipids from a 20 gram lipid sample. The recoveries including instrumental variation are usually between 65-90%. The method described above is validated against the old modified[4] method used in our laboratory. Results from repeated single analyses of the same fish homogenate with the old method (A, B, C, D) and the new method (E, F) is presented. The results are in very good agreement with each other. The man-hours needed in the new method is approximately the same and the use of chlorinated solvents is reduced. This is, in fact, timesaving considering the possibility to include additional analyses of non-ortho and mono-ortho PCBs from the same extract. The SPM can also be an excellent step in removing other large molecules from different kind of samples like sediments, low quality paper products and tar containing products.

References

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