New rapid clean-up method for PCDDs and PCDFs determination in milk and eggs samples.

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It is reasonable to begin the estimation of a certain region contamination by PCDDs/PCDFs with the analyses of the most informative matrices. When agricultural regions are investigated, cow milk, butter and eggs accumulating dioxins are suitable for this purpose. The human milk is one of the most informative matrices for estimation of human exposure by PCDDs/PCDF. However, taking into account the differences betwen social status of the women, the human milk samples should be thoroughly averaged.

After the carbon microcolumn designing [1,2] a cleanup procedure for PCDDs/PCDFs determination in butter was significantly shortened and simplified. Unfortunately, cleanup procedures for milk and eggs used for the present time are very laborious and time consuming [3-6]. Lipid extraction is a common initial step of the sample preparation for the analysis of PCDD/PCDF. It is the most difficult step due to ethylether using, emulsion formation and slow separation into layers, when liquid-liquid extraction takes place. It makes difficult to use milk and eggs for rapid dioxin control.

A new rapid cleanup technique for the isolation of PCDDs/PCDFs from milk and eggs is suggested. This method allows us to make sampling procedure faster, more effective and less expensive.

We use extraction with water miscible solvent (acetone) followed by the saturation with ammonium sulphate. The extraction proceeds for a short time without emulsion formation. In addition, proteins are precipitated by ammonium sulphate, the grain sediment is easily filtrated or centrifuged. PCDDs/PCDFs are isolated from this acetone extract by applying of carbon microcolumn. The microcolumns described previously were packed with activated carbon AX-21 or FAS. PCDDs/PCDFs were eluted with 100 ml of toluene from these columns. A new activated carbon FAS-MD is used in this work. It was prepared specially for the isolation of dioxin and dioxin-like compounds. In this case, only 5 ml of toluene elute all PCDDs/PCDFs from the carbon microcolumn.

For the following cleaning a very small "multilayer" column is needed, because not more than 5 mg of matrix are eluted by toluene from the carbon microcolumn. It is not necessary to evaporate toluene eluate because the cleaning on the "multilayer" column is well enough in hexan-toluene (9:1). The "multilayer" column is rinsed with an equal volume of hexane, so that toluene concentration in hexane becomes c.5%. Such concentration of toluene does not prevent PCDDs/PCDFs from being retained by Al_2O_3. Cleanup procedure with Al_2O_3 goes as usual[7]. The rest of toluene is removed with the first (hexane) and the second (hexane-methylenechloride, 95:5) fractions, so that PCDDs/PCDFs fraction (hexane-methylenechloride,50:50) can be easily evaporated.

Experimental section

Reagents and Apparatus: All solvents and reagents were Pesticide Grade (Burdick & Jackson) and "puriss" (RUS); Kieselgel 60 (Merck); Al_2O_3 (Bio-Rad); activated carbon FAS-MD (Institute of physical chemistry, RUS, Laboratory of sorbents synthesis and investigation, Polyakov N.S.); $^{13}C_{12}$- and $^{13}C_6$-labeled PCDD standards (CIL). Homogenizer ULTRA-TURRAX 125 (Janke & Kunkel IKA-labortechnik). The GC/MS determinations were performed on gas chromatograph Varian 3400 and high resolution mass spectrometer Finnigan MAT HSQ-30. A 30-m HP-17 fused silica capillary column with 0.25 mm film thickness was employed.

Procedure: Milk extraction.

A 150 ml sample of cow milk is spiked with a standard mixture containing 2 ng $^{13}C_{12}$TCDD, 2 ng $^{13}C_{12}$PCDD, 4 ng $^{13}C_{12}$HxCDD, 4 ng $^{13}C_{12}$HpCDD, 4 ng $^{13}C_{12}$OCDD and equilibrated by gentle shaking. The sample is mixed with 150 ml of acetone and 150 ml of hexane and swirled by homogenizer for 3 min till homogeneous medium is formed. Then 105 g of ammonium sulphate are added. The mixture is shaked till all ammonium sulphate is dissolved. When the layers are separated (5 min), the sediment is filtrated through glass filter with a gentle vacuum. The sediment is washed with two 10-ml portions of hexane-acetone (1:1). Two hexane-acetone rinses are added to the main extract. The organic layer (c.300 ml) is isolated with the separatory funnel; 20 ml of acetone are added. The aqueous layer is discarded.

Eggs extraction.

One egg is weighted, water is added up to 200 g and mixed by homogenizer for 5 min. The mixture is spiked with $^{13}C_{12}$-labeled standards as described above. The spiked sample is homogenized with 200 ml of acetone and 200 ml of hexane for 3 min. 150 g of ammonium sulphate are added. The mixture is shaked till all ammonium sulphate is dissolved. After the separation into layers (c.1 hour) the organic layer is decanted. The remainder is centrifuged (3000 rpm, 5 min) and organic layer is decanted. The organic extracts are combined; 20 ml of acetone are added. The total volume of organic extract is about 400 ml.

Carbon microcolumn.

The carbon microcolumn is packed with 20 mg activated carbon FAS-MD on 200 mg Celite. The extract is applied to the carbon column with a flow rate of ~2 ml/min. The column is washed with 20 ml of hexane-acetone (1:1). PCDDs/PCDFs are eluted in the reverse direction with 5 ml of toluene at 80°C. The hexane-acetone extract is evaporated, the total lipids are determined gravimetrically.

"Multilayer" column.

The sample eluate in toluene (5 ml) is mixed with 45 ml of hexane. This solution is applied to "multilayer" column (ID=10 mm) packed with 1 cm^3 of silica neutral, 1 cm^3 of 40% H_2SO_4/silica, 1 cm^3 of K_2SiO_3/silica, 1 cm^3 Na_2SO_4 (from top to bottom). 50 ml of hexane are added through this column.

Neutral Aluminia Column.

Eluate (c.100 ml) is applyed to the column packed with 4 g of neutral aluminia. Interferences are eluted with 20 ml of hexane and 30 ml of methylene chloride/hexane (5:95) sequentially. Analytes are then eluted with methylene chloride/hexane (50:50). $^{13}C_6$TCDD is added as internal standards and solvent is concentrated and transferred into a small sample tube containing 5 ml of tridecane as a keeper. The solvents are removed under a stream of nitrogen.

Results and Discussion

1. The recoveries of ^{13}C-labeled PCDDs from spiked milk and egg samples are presented in Table I.

Table I. Percent Recoveries of $^{13}C_{12}$-congeners from spiked milk and egg samples (mean and standard deviation; n=4)

Component	milk	eggs
$^{13}C_{12}$TCDD	68+10	64+12
$^{13}C_{12}$PCDD	55+14	62+10
$^{13}C_{12}$HxCDD	67+8	50+12
$^{13}C_{12}$HpCDD	50+5	55+7
$^{13}C_{12}$OCDD	60+9	57+13

2. The suggested method makes sample treatment procedure for dioxin analysis in milk and eggs significantly simpler, faster and cheaper. It allows to use less quantities of the sorbents and the toxical solvents.

3. The procedure rans without evaporations - "on line". It can be easily automated.

References.

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