OPTIMIZED MANUAL SAMPLE PREPARATION METHOD FOR DIOXIN ANALYSIS.

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Introduction

It is well known, that the analysis of PCDD/Fs and related compounds in environmental and biological samples is a complex and expensive task. The demand for this type of trace analysis still increase since more and more countries discover environmental problems. Recently several commercial or well-financed laboratories applied automatic sample preparation systems and are using subcritical extraction technique (ASE). We do not want to prejudice the advantages of this systems, but their use can be inaccessible or unprofitable for scientific or low budget laboratories. In the present work we want to demonstrate that the PCDD/PCDF clean-up can be performed in a scientific laboratory with moderate effort (e.g. usual labware) and anyway provide sufficient efficiency for the following GC-MS analysis.

Extraction

Biological samples

All type of biological samples can be extracted by the salt-out method. This old-established method is based on the ability of water-soluble inorganic salts to separate lipophilic substances from aqueous phase. The best results were achieved using ammonium sulfate. Three layers are formed during this extraction – aqueous, liquid organics (solvent, fat and others lipophilic compounds) and coagulated proteins between them. The technique of PCDD/Fs extraction from fish sample are as follows:

- 100 g fish was ground in a mincing machine. The sample was placed in a 500 ml flask and spiked with ^{13}C-labeled PCDD/F standard mixture. After the addition of 150 ml of acetone, the flask was placed in an ultrasonic bath. After 10-15 min 150 ml of hexane is added and sonicated for 10 min [NOTE for this time the sample absorbed the solvent and swelled]. Then 60 g of ammonium sulfate are added [NOTE it is necessary to obtain a saturated solution (71 %)]. The sample was mixed by a homogenizer within 30 minutes. After 1-2 hours the top fraction was decanted and filtered (if it is necessary) through sodium (or magnesium) sulfate in a funnel [NOTE the use of this method does not result always in an instant phases separation]. The residue was washed two times by 30-50 ml of hexane. The consolidated extract cleared as described below.

Other biological samples (meat, egg, milk, butter, serum, whole blood) can be extracted similarly to the fish samples. The fat percentage can be easily determined by gravimetrici after applying a carbon column.

Solid samples

Almost all types of soils, sediments, analytic-resins, air filters with soot [NOTE with the exception of fly ash] can be extracted in a continuous-flow extractor. As well as in ASE, the high efficiency of extraction is reached by continuous passing of the pure hot solvent through thea sample. This result in

an increase of solubility and provides a high concentration gradient for the extractable substance. The extraction is carried out at a temperature, which is not exceeding the boiling point of the solvent (or high-boiling solvent mix). The pressure in the system does not exceed 2 atm. The described method is not limited to PCDD/F extraction but can be used for the recovery of different types of organic substances. For the extraction of dioxins and others lipophilic compounds is rational to use toluene or a solvent mix: e.g. hexane-acetone-i-octane (45:50:5) [NOTE i-octane serves as keeper in the following evaporation], or toluene-acetone (75:25). The latter mix at 98 °C is the most effective solvent for man-caused matrixes. The use of acetone allow to prevent drying the samples (for example filters) if it is not necessary to know their exact weight. The technique of PCDD/Fs extraction from industrial sediments are given below, Other samples can be extracted similarly.

• An Industrial sediment sample was dried at 45-50 °C and milled up to particle size <0.,25 mm. 10 g of the sample was transferred to the extraction cell (2) and spiked with a PCDD/Fs $^{13}C_{12}$-labeled standard mixture. The cartridge was screwed together with the heat exchanger (1). 200 ml of toluene-acetone mix (75:25) were fed continuously by inert gas pressure (0.2-2 atm) from a reservoir, connected with a fitting (10) by a teflon tube, [NOTE can be used special reservoir or bottle from sparkling wine]. A flow rate of 15-25 ml/min and thermostat temperature 98 ºC were used.

Depending on the amount of the sample various cells volumes 10, 30, 60, 150 cm^3 can be chosen. Solvent volume can be selected depending of the type of sample. Usually we used 200 ml for 10 g sample (dry weight) and 350 ml for 20-25 g respectively. Air or stock gas samples (100 cm^3 XAD-2 and filters) can be extracted by 750-1000 ml of solvent. The main advantages of the given method are: speed, compactness, safety, low price, absence of glass details, easy cleaning.

The contentious-flow extraction as salt-out method can be used in addition to PCDD/F also for PCB, PAH and other aromatic? organic? compounds.

Fly ash

As usual, fly ash extraction includes an acid treatment and toluene extraction. We propose to combine the two steps:. Hydrochloric acid and toluene can form a homogeneous solution by the addition ofIn ethylene glycol dimethyl ether. After the extraction, ethylene glycol dimethyl ether, and HCL can be separated from the toluene (and the extracted organics) by dilution in water.

• Fly ash sample (up to 10 g) was placed in at round-bottom flask (500 ml) ,and spiked with a PCDD/Fs $^{13}C_{12}$-labeled standard mixture. 70 ml toluene, 10 ml of HCl and 50 ml of ethylene glycol dimethyl ether were added to the fly ash fla The mixture was boiled under reflux over night. After cooling down, 300 ml of distilled water was added and the toluenec phase was separated. The residue was washed two times by 30-50 ml of toluene. The toluene fractions were combined and the consolidated extract cleared as described below.

Clean-up

Washing of glassware is one of the problems associated with dioxin clean-up. Therefore we tried to reduce the quantity of reentrant glasses.

The proposed arrangement of the samples clean-up is universal for all kinds of samples and scan be carried out on identical glassware and equipment [NOTE regardless of the fact that the identical glassware is used, it is expedient to use different complete sets for different types of matrixes]. For clean-up procedure necessary and sufficient: pear-shaped flack (250 ml) for rotary evaporator; column (for multilayer and alumina) with ground glass joints combined with one reservoir and a glass funnel. Further, disposable glass pipe (4 mm id, 6 cm long), Pasteur pipette, 5 ml vial and empty bottle from solvent or equivalent. The applied columns and the quantity of acid silica in the multilayer column can be adjusted to the type of sample (tab. 1).

Table 1. Dependence of the clean-up stages from samples type.

Biological samples	Clean samples (soils with low organic carbon content, etc.)	Dirty samples
Carbon column Multilayer column Alumina column	Multilayer column Carbon column Alumina column	Hot multilayer column Carbon column Multilayer column Alumina column

Carbon column

Contained a mixture of 20 mg carbon AX-21 (fractional precipitation in methanol, 10 min) and 180 mg Celite (C-22, 60-80 mesh) fixed between glass-wool plugs (Whatman) in a glass pipe (4 mm id).. The column is connected with 10 ml screw capped reservoir (teflon thick pipe) by a teflon tube, and with a small teflon pipe on the other side. The column was washed by 5 ml toluene before use., (how was the sample applied? which is the order of the fractions**?): For the extraction, 2 ml toluene-DCM (1:1), 2 ml hexane-DCM (1:1) and 5 ml hexane are used. Toluene must not be contained in purified extract. Thus if toluene was used for the extraction, the solvent must be rotary evaporated near dryness and, the sample dissolved in 5 ml dichloromethane (DCM) or other non-aromatic solvent. Volumes of biological extract are varied with the corresponding fat percentage, i.e. solution viscosity. Next prepared sample was passed through carbon column; the pear-shaped flack was washed two times with 5-10 ml DCM under sonication and the solvent was transferred to the column. Next column was rotated, placed to the oven and eluted with 10 ml toluene at 100 °C in back-flow.

The solution which passed through the column in forward flow can be used for the analysis in the non-planar compounds or for the determination of the fat content by gravimetric.

Earlier we reported [1] about the application of a similar column, with Russian carbon – FAS-MD. This carbon in comparison to AX-21 provides 15 % higher recovery for higher-chlorinated congeners. Unfortunately it contains fair quantity of PAH compounds requiring the use of a multilayer column after the carbon column and can result in elevated noise in the GC/MS measurement (especially for LRMS).

Hot multilayer column

Achieve high clean-up quality compared to other column. Under elevated temperatures, many relative inert compounds are oxidized on acid silica. For example toluene and i-octane at 95 ºC caused a strong coloration of column. Therefore it is necessary to use n-alkane or chlorinated solvents. We propose to use chloroform for transferring the sample to the column and hexane as mobile phase at column temperature of -approximately 60 ºC. The dioxin fraction is eluted by DCM-hexane (1:3 v:v) under room temperature. The cleaning-up of stack gas samples is given as example:.

• The extracts were rotary evaporated to 0.0,.5-1 ml. ,The residual (which usually is a viscous brown liquid) was dissolved in 5 ml chloroform and transferred to the hot multilayer column (35 cm long, 16 mm id., placed in water heater-jack and connected by ground glass joints with a (solvent?) reservoir). The column consisted of following layers, (from top to bottom) -: 2 cm^3 K_2SiO_3, 1.5 cm^3 $MgSO_4$, 5 cm^3 30 % H_2SO_4/SiO_2, 1.5 cm^3 $MgSO_4$, 10 cm^3 40 % H_2SO_4/SiO_2, 1.5 cm^3 $MgSO_4$, 15 cm^3 44 % H_2SO_4/SiO_2, 1.5 cm^3 $MgSO_4$, 7 cm^3 K_2SiO_3 and 1.5 cm^3 $MgSO_4$, [Note: The sequence of the layer avoid a strong carbonization of the sample in the upper part of the column; it is necessary to use

magnesium sulfate sinced sodium sulfate lost water under the elevated temperature]. The pear-shaped flack was washed two times with 4 ml DCM under sonication, with solvent transferring to the column. Next flack placed under the column for extract collection and 100 ml of hexane was added to the reservoir. Elution was carried out under small inert gas pressure (0.0,1 atm). Then all solutions are passed through the column. Finally the column is removed from the heater-jack and washed with 75 ml hexane:DCM (3:1 v:v)

Alumina column is used as lastthe final step of our purification procedure.

• The solution was rotary evaporated to 0.5 ml and transferred to the alumina column (4g basic alumina, activated overnight at 580-600 ºC). The pear-shaped flack was washed two times consistently with 10 ml of hexane and 20 ml hexane:DCM (95:5 v:v). The pear-shaped flack was washed with K2Cr2O7/H2SO4, distilled water and acetone washed while the solvent mix was passed though the column. Finally the column was eluted with 50 ml hexane:DCM mixtutre (40:60 v:v).

For serial analysis we processed 5 samples in parallel. With the shift of stages we could simultaneous process two series –making elution without pressure in the beginning and end of a working day??. In this case two employees can prepare up to 25 samples per week.

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References

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