# Simultaneous Determination of Benzo(a)pyrene and Dioxins in the Food Samples Using New Rapid Clean-up Procedure

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## Abstract

The new easy rapid technique for simultaneous determination of the most hazardous from polyaromatic hydrocarbons benzo(a)pyrene and PCDDs and PCDFs in complex lipophylic matrices is developed. Generally, this method consists of efficient extraction involving salting out procedure followed by clean-up on the carbon microcolumn that permits to separate from fats and lipids without many-stages liquid-liquid extraction, after final purification quantitative definition of content of this substances provided by HPLC for benzo(a)pyrene and GH-MSHR for PCDDs/PCDFs. The results of determination of benzo(a)pyrene in some kinds popular in Russia food are presented.

## Introduction

The global problem of the strong environmental pollution by polycyclic aromatic compounds that are formed in the results of incomplete combustion of organic matter is obvious. Many of such pollutants, being included in the environmental on the trace level, have direct or potential mutagenic and/or carcinogenic activity and therefore may be called "superecotoxicants". Among of them polycyclic aromatic hydrocarbons (PAHs) are presented widely. Well-known, that benzo(a)pyrene is the most dangerous from polycyclic aromatic hydrocarbons. In Russia created limited concentration of benzo(a)pyrene content in the environment is follow: 0.1 $\mu g/m^3$ in air, 5 ng/l in drinking water, 20 $\mu g/kg$ in soil; also it is recommended to define benzo(a)pyrene in food: vegetates and vegetable oil, milk, bread, boiled and smoked sausages, smoked fish.

Due to widespread of "superecotoxicants" in trace amounts (on the *ppb* level) the existent technics of its determination in the complex matrices as food usually require to provide continuous many-stage analysis. Earlier by our group the new rapid, fairly simple method of definition of PCDDs and PCDFs in the lipophylic matrices was made[1-3], which was underlain of the presented one. Its essential part consists on using both efficient extraction with simultaneous saturation of fats and easy clean-up procedure on the carbon microcolumn that permit to exclude usually provided to remove lipids many-times liquid-liquid extraction

from the scheme of analysis. Using this technique, content of 10 priority polyaromatic hydrocarbons was determined in cow milk and butter[4].

The purpose of our work was to survey of benzo(a)pyrene (BP) content in some kinds of food that are popular in our country. It is a single polyaromatic hydrocarbon for which recommended limited concentration in food is created by Department of Health Protection in Russia. For this investigation one sample of the boiled sausage was taken from Ufa, all other foods was commercially available in the market in Moscow.

**Experimental Methods**

Reagents and Apparatus

All solvents and reagents were Pesticide Grade (Burdick & Jackson) and "puriss" (RUS); $Al_2O_3$ 1 Brockman activity (Alltech Associates); Activated carbon FAS-MD (Institute of physical chemistry, RUS, Laboratory of sorbents synthesis and investigation, Polyakof N.S.); Celite 545 (Altech associates); Standard solutions of benzo(a)pyrene and 1,1`-binaphtyl (Serva); The solid-phase octadecyl bonded cartridge (C18, 200 mg, Altech associates); Homogenizer ULTRA-TURRAX 125 (Janke & Kunkel IKA-labortechnik). Determination of benzo(a)pyrene were performed at HPLC HP 1090 with programmable fluorescence detector model 1046 A. Zorbax ODS (250:4,6 mm) column was employed with flow rate 1,5 ml/min, oven temperature 40°C.

Sample extraction

Each sample was analyzed both blank and with addition of benzo(a)pyrene before extraction to estimate the recovery percent provided by the new technique.

*Butter, Margarine*

10 g sample of butter (or margarine) was solved in 200 ml of hexane/acetone mixture (1:1) on the ultrasonic bath, 20-50 g of ammonium sulfate were added and shacked with solution during 5-10 min and then left on warm water bath (40° C) for better settled. After then organic layer was decanted and filtered.

*Cow milk*

100 ml of milk was mixed with 100 ml of acetone and 100 ml of hexane were added and swirled by homogenizer for 3 min till homogeneous medium was formed. After addition of 70 g of ammonium sulfate the mixture was shacked till all ammonium sulfate was dissolved. When the layers separated (5 min), the sediment was filtered through glass filter with a gentle vacuum. The sediment was washed two times 10 ml of hexane-acetone (1:1). Two hexane-acetone rinses were added to the main extract. The organic layer (c.300 ml) was isolated with the separatory funnel; 20 ml of acetone were added. The aqueous layer were discarded.

*Bread*

The cutted sample of white wheat bread (50 g) was shaked with 150 ml of acetone and 150 ml of hexane and 20 g of ammonium sulfate by homogenizator. After faint heating on the warm water bath (40° C) for better dissolving of inorganic salt extract was filtered through funnel

with glass filter bottom and solid residue was washing two rinses by hexane-acetone (1:1) which then were added to the main extract.

*Sausage*

Samples of the all kind of sausages were extracted identically. After rough homogenization 50 g (or 46 g) sample of sausage were shaked vigorously with 150 ml of acetone and then 150 ml of hexane and 50 g of ammonium sulfate by homogenizator (in the case 25 g samples 75 ml of acetone and then 75 ml of hexane and 25 g of ammonium sulfate were added). After extraction flask were heating faintly on the warm water bath (40° C) for better settled extract was filtered through funnel with glass filter bottom. The organic layer was isolated with the separatory funnel; 20 ml of acetone were added. The aqueous layer were discarded and solid residue was washing two rinses by hexane-acetone (1:1) which then were added to the main extract.

Carbon microcolumn

The carbon microcolumn packed with 20 mg of activated carbon FAS-MD on 200 mg Celite was produced from glass pipette (ID=3,5 mm). The length of sorbent layer was 2,5 cm. Sorbent was fixed at two ends by glass fiber plugs. The extract was applied to the carbon column under a pressure of 0,5-1,5 atmospheres. The column was washed with 20ml of hexane-acetone (1:1). Benzo(a)pyrene were eluted in the reverse direction with 10 ml of toluene at 80$^0$C. Heating proceed by use of microstove made from resistor (15 RUS).

Basic Alumina Column

For further operation it is enough only 5 ml of toluene extract. Other aliquot can be used for PCDD and PCDF analyses. 5 ml of toluene eluate was mixed with 95 ml of hexane and applied to the basic alumina column with 4 g of sorbent. The column was washed with 20 ml of hexane, 30 ml of methylene chloride-hexane (5:95), 50 ml of methylene chloride-hexane (50:50) and 50 ml of methylene chloride-methanol (50:50), sequentially. 100 ng of binaphtyl was added as internal standard. The methylene chloride-hexane (50:50) fractions were collected and evaporated on the vacuum gauge under heating to 40$^0$C. The residue was dissolved in 1 ml of acetonitrile.

Octadecyl Bond Cartridge

C18 cartridges were used to minimize the noise level for all samples. Before use the C18 cartridges were activated with 2 ml of methanol, 2 ml of deionized water and 2 ml of acetonotrile, sequentially. The sample was passed through an activated C18 cartridge in 1 ml of acetonitrile.

**Results and Discussion**

Two main advantages of presented methods, the scheme is shown at the Fig.1, are ease of extraction of benzo(a)pyrene from complex lipophilic matrices and new express procedure for removal fats and lipids, that permits to manage do with-out prolonged many-times liquid-liquid extraction required by the traditional methods. It was shown that while more than 99,9% of lipids pass through the carbon microcolumn benzo(a)pyrene as all early studied PAHs sorb in it from the acetone-hexane solution. Several kinds of activated carbons were

investigate to concentrate benzo(a)pyrene, the best from them is occurred FAS-MD. The elution of BP occurs best by 10 ml of toluene heated to $80^0$C that agree to data obtained early for PAHs and PCDDs/PCDFs.

Tree types of alumina was tested for the next clearing of the extract (neutral, basic and acidic). The column packed with 4 g of basic alumina was appeared the best for the isolation of interferences from benzo(a)pyrene. Benzo(a)pyrene was eluted from this column with 50% $CH_2Cl_2$:hexane without breakthrough in previous fractions.

Applying of the C18 cartridges allowed to decrease of a noise level for all studied samples significantly and therefore to provide HPLC analysis.

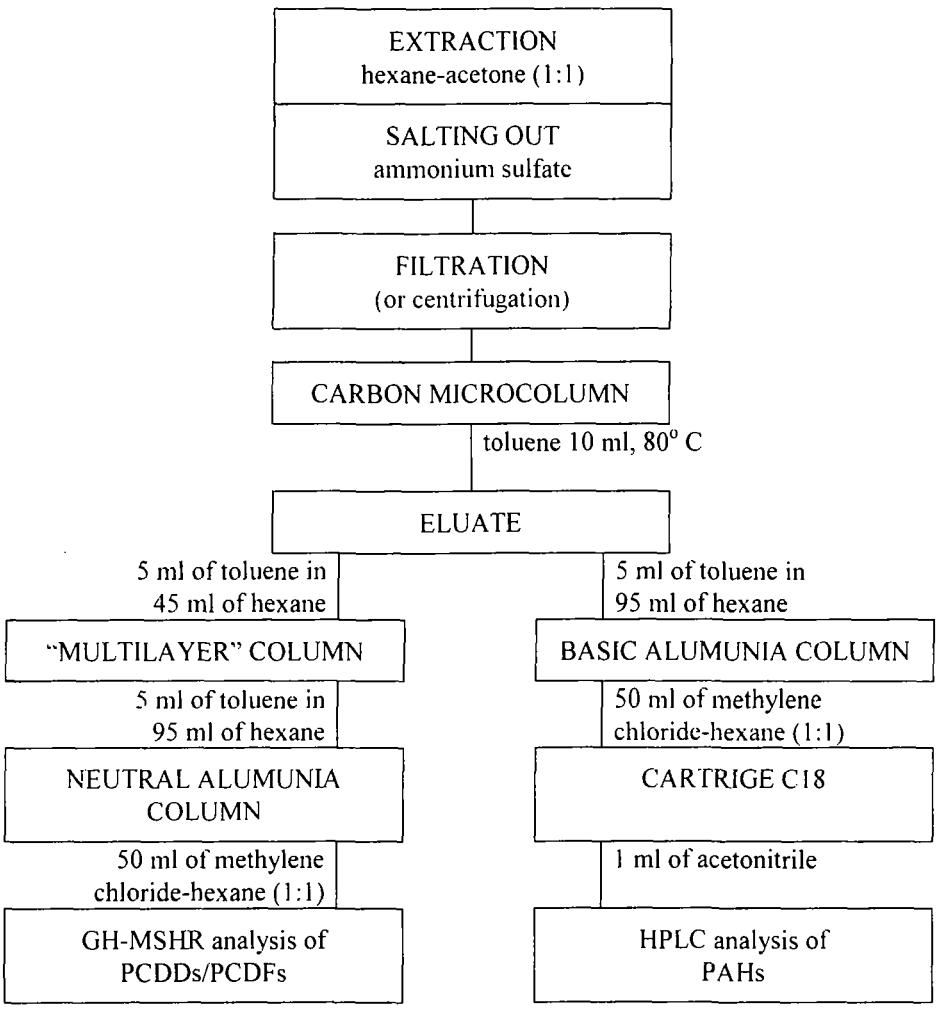


Figure 1. Sample preparation for simultaneous analysis of PCDDs/PCDFs and PAHs in lipophilic matrices.

As shown at the Table 1, percent recoveries of benzo(a)pyrene ranged from 40 to 78%. The blank domestic and imported food samples of milk, butter and margarine, broad, some sausages contained traces of BP, but, as shown in Table 1, on the lower level that is recommended limited concentration (LC) of benzo(a)pyrene recommended by Department of Health Protection of Russia. Two kinds of domestic sausages considered free from benzo(a)pyrene. It is should be noted, that there is possible to decrease amount of sample to be analyzed.

Table 1.

Results of determination of benzo(a)pyrene in food popular in Russia.

| Food | Made in | Sample | Percent Recoveries of BP, % | Found in sample BP, μg | Content of BP, μg/kg | LC* of BP, μg/kg |
|---|---|---|---|---|---|---|
| Milk | Russia | 100 ml | 64±3 | 0.0076± ±0.0004 | 0.118± ±0.005 | 0.13 |
| Butter | Russia | 10 g | 63±2 | 0.0062± ±0.0002 | 0.980± ±0.030 | does not created |
| Margarine | Russia | 10 g | 50 | 0.0104 | 2.159 | does not created |
| Bread | Russia | 50 g | 57 | 0.0081 | 0.281 | 0.15 |
| Sausage smoked | Germany | 10 g | 68 | 0.0008 | 0.049 | 1.1-3.0 |
| Sausage semismoked | Danish | 25 g | 78 | 0.0110 | 0.283 | 16.5-29.5 |
| Sausage semismoked | Russia | 50 g | 76 | --** | -- | 16.5-29.5 |
| Sausage boiled | Russia | 50 g | 71 | 0.0077 | 0.216 | 0.40-0.66 |
| Sausage boiled | Russia | 46 g | 40 | -- | -- | 0.40-0.66 |

* Limited concentration (LC) of benzo(a)pyrene recommended by Department of Health Protection. Russia

** benzo(a)pyrene was not found

**Conclusion**

The cleanup procedure for the analysis of benzo(a)pyrene together with PCDDs/PCDFs in lipophilic matrices provides an effective results for monitoring a large number of samples. The new method requires relatively small quantities of expensive and hazardous solvent and make the clean-up for lipophylic matrices cost-effective and fast. Moreover, it allows to minimize the cross contaminations from high-levels samples. The clearing quality and average recovery percent comparable with standard method. Presented

technique allows to determine benzo(a)pyrene on the level lower that recommended limited concentration.

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