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RECOMMENDED METHOD FOR A THIN-LAYER-CHROMATOGRAPHIC SCREENING METHOD FOR THE DETERMINATION OF BENZO(a)PYRENE IN SMOKED FOOD

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Recommended method for a thin-layer-chromatographic screening method for the determination of benzo(a)pyrene in smoked food

Abstract - Results are reported of a collaborative study on the determination of benzo(a)pyrene in smoked meat. To this end four meat samples were analyzed for concentrations < or > 0.6 µg benzo(a)pyrene/kg sample.

Polycyclic aromatic hydrocarbons (PAH) are a well known class of environmental carcinogens and in the past benzo(a)pyrene (BaP) often has been used as an indicator for their presence and even as a numerical parameter for the carcinogenic potency of environmental matter, although numerous other PAH also exhibit a comparable biological activity in various systems. This has lead to national regulations in some countries (1). For instance, in FRG a concentration of 1 µg BaP/kg has been set to be the upper limit in smoked meat. Accordingly, a rapid method is required to check smoked food for its benzo(a)pyrene concentration. A method previously described (2) for the determination of BaP, based on thin-layer-chromatographical comparison of BaP of a meat extract with authentic BaP-concentrations, has been optimized and applied in an international exercise by several laboratories.

SCOPE AND FIELD OF APPLICATION

This method specifies a procedure for the rapid determination of benzo(a)pyrene in smoked meat with the intention to select samples containing concentrations of more than 0.6 µg BaP/kg. It also can be applied to other materials. The enrichment procedure specifically separates PAH and subsequent thin-layer-chromatography results in a good separation of BaP.

The method has a limit of detection of 0.5 ng BaP.

PRINCIPLE

The sample is grinded in a mincer (meatchopper) and homogenized for 20 sec at room temperature in 1,1,2-trichlorotrifluorethane (TCFE) or, alternatively, refluxed for 30 min with TCFE or cyclohexane. An aliquot of this solution obtained by decanting is extracted by methanol/water (7+3). In case of TCFE extraction this is removed by distillation and the residue then is dissolved in cyclohexane, which is not required in case of extraction with cyclohexane. The cyclohexane is extracted with a mixture of N,N-dimethylformamide (DMF)/ water (9+1). The remaining DMF-water phase is diluted with water and reextracted with cyclohexane. After concentration to 1 ml it is filtered through silica. After solvent evaporation the residue is redissolved in a small amount of cyclohexane (60 µl). 10 µl as well as 20 µl of this solution are compared by thin-layer-chromatography on acetylcellulose or on mixed plates with definite BaP-amounts, respectively, using the system acetone/ ethanol/ water (25+60+15) for elution and fluorescence (excitation wavelength 366 nm) for detection.

A schematic representation of the procedure is shown in Fig. 1.

MATERIAL

All chemicals used were of analytical grade and were checked for BaP-contaminations. Solvents were freshly redistilled in carefully cleaned glass vessels. The BaP-standard solution content was 1 ng BaP/µl in cyclohexane. BaP in a purity > 99.0 % was obtained from the Commission of the European Community, Community Bureau of Reference, BCR, Rue de la Loi 200, B-1049 Brussels, Belgium. The solution must be kept in darkness and should not be used for longer than one month.

Silica filtration was carried out with silica 60 (particle size 0.0630-200 mm ≙ 70-230 mesh ASTM). It was desactivated with 10 % water 24 hours before used. For TLC commercially available or self-made thin-layer plates coated with acetylated cellulose or acetylated cellulose + alumina were used.

To check the absence of BaP-contaminations a blank test should be carried out with the whole procedure.

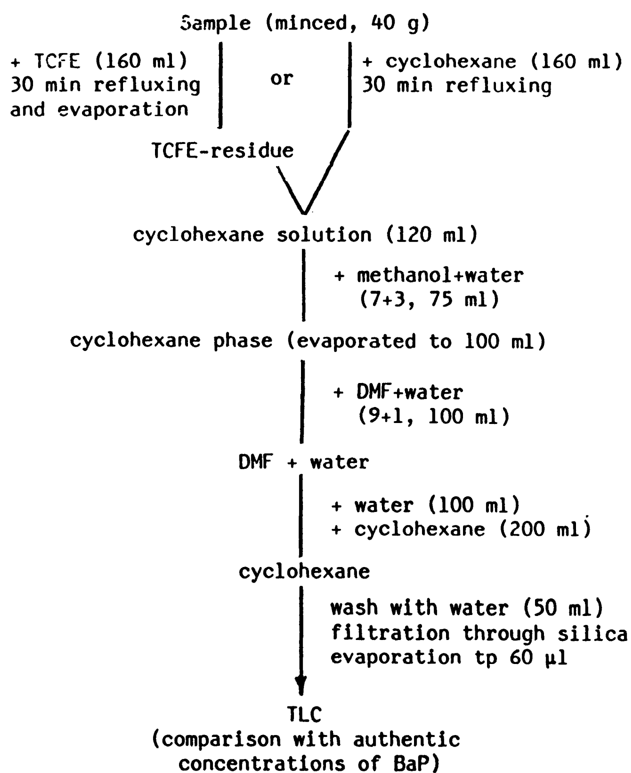

Fig. 1. Schematic representation of the procedure
TCFE: 1,1,2-trichlorotrifluorethane;
DMF: N,N-dimethylformamide;
TLC: thin-layer-chromatography).

TABLE 1. Results of the TLC screening determination of BaP in meat samples.

Laboratory	A-1	A-2	A-3	A-4
1	1	1	1	1
2	1	1	1	1
3	1	1	m	m
4	1	1	1	m
5	1	1	1	1
6	1	?	m	?
7	1	1	1	1/1**
8	-	-	m	1
9	-	-	1	1
10a*	-	-	1	1
10b*	-	-	1	1
11	-	-	1	1
12	-	-	1	1
13	-	-	1	?
14	-	-	1	1

1 = <0.6 µg BaP/kg;
m = >0.6 µg BaP/kg
? = no answer obtained
- = no samples distributed to these laboratories
* = same laboratory, different operators
** = duplicate determination

Four commercial samples of smoked ham were distributed (A-1; A-2; A-3 and A-4). Before distribution the samples were analyzed for their BaP-content by capillary gas chromatography; the following concentrations were found: A-1 and A-2 (about 0.1 µg BaP/kg); A-3 and A-4 (about 0.4 µg BaP/kg). A-1/A-2 and A-3/A-4, respectively were identical samples.

DESCRIPTION OF THE PROCEDURE

1. Extraction

1.1 The sample (about 250-500 g) is homogenized by a mincer. 40 g of this sample are weighed in a vessel suitable for homogenization by an ULTRA TURRAX (or comparable instrument) or in a 250 ml round bottom flask and covered with TCFE (160 ml) (TCFE can be replaced by cyclohexane).

1.2 The mixture is homogenized for 20 sec at room temperature or refluxed for 30 min if no homogenizer is available.

1.2.1 In case of TCFE-extraction the solution is cooled to room temperature, the supernatant is decanted and an aliquot (120 ml) is evaporated to near dryness using a rotatory evaporator (reduced pressure, water bath temperature 35 ^{o}C). Then it is redissolved in 120 ml cyclohexane.

1.2.2 In case of cyclohexane extraction the solution is cooled to room temperature and an aliquot of 120 ml ($\hat{=}$ 30 g sample) is taken.

1.3 The cyclohexane-aliquot (120 ml from 1.2.1 or 1.2.2, respectively) is transferred into a separation funnel and extracted with a mixture of methanol and water (7+3), 75 ml to avoid formation of emulsions during the following liquid-liquid partition.

1.4 The methanol-water phase is discarded. The cyclohexane-phase (120 ml) is evaporated to 100 ml, using a rotatory evaporator (reduced pressure; water bath temperature of 35 ^{o}C).

2. Liquid-liquid partition

2.1 N,N-dimethylformamide (90 ml) and water (10 ml) are added to the cyclohexane solution (100 ml) and the mixture is shaken (about 5 min) then allowed to separate and the upper layer (cyclohexane) discarded.

2.2 Water (100 ml) and cyclohexane (200 ml) are added to the DMF-water phase (from 2.1), the mixture shaken for about 5 min and allowed to separate. The lower layer (DMF-water) is discarded.
2.3 The cyclohexane layer is washed with water (50 ml).
2.4 The cyclohexane solution is concentrated under reduced pressure to about 3 ml, using a rotatory evaporator (water bath temperature 35 °C).

3. Chromatography on silica column
3.1 A silica column is prepared by adding 40 ml cyclohexane to 10 g silica. The mixture is shaken to degas, and the slurry poured into the glass column and the solvent run off. The slurry is rinsed from the wall of the column, the solvent run off again until liquid level coincides with the upper surface of the silica gel.
3.2 The concentrated extract (2.4) is transferred to the top of the column, the evaporation flask rinsed with cyclohexane which is also transferred to the column. Extract and rinsing is allowed to run down the column, until solvent coincides with the adsorbent layer.
3.3 The column is eluted with 140 ml cyclohexane.
3.4 Eluate is evaporated to near dryness, using a rotatory evaporator under reduced pressure to maintain boiling (bath temperature of 35 °C).
3.5 The residue is dissolved in acetone (about 1ml)

4. Concentration for thin-layer chromatography
4.1 The solution (3.5) is transferred to a concentration tube and the round bottom flask (3.4) rinsed again with acetone (1 ml) and the solution added to the same concentration tube.
4.2 Acetone is evaporated to near dryness, using a rotatory evaporator, taking care to avoid splashing to the walls of the concentration tube.
4.3 Using a rotatory evaporator, it is necessary to rinse the wall of the tube with acetone (0.5 ml) again. Repeat 4.2.
4.4 The residue of the tube is dissolved in 60 µl cyclohexane right before dropping onto the TLC plate.

5. Thin-layer chromatography
5.1 Preparation of the developing tank: acetone, ethanol, and water (25+60+15) are mixed and poured in the developing tank which is saturated by the solvent mixture during several hours.
5.2 A line, 1 cm from one end of the (precoated) plate, is marked for the starting points.
5.3 On this line, 20 µl of the sample solution (= 10 g of the sample), 6 µl of the BaP-solution (= 6 ng BaP), 10 µl of the sample solution (= 5 g of the sample), as well as 3 µl of the reference solution (= 3 ng BaP) are applied using a syringe. Damaging the surface of the coated plate carefully has to be avoided.
5.4 The plate is placed in the developing tank.
5.5 After a distance of 10 cm, the run is finished, and the plate is dried at room temperature.
5.6 The run is repeated beginning at 5.4.
5.7 The degree of intensities of the fluorescence (366 nm) of the spots from the sample and from BaP are compared.
5.8 The content of benzo(a)pyrene is estimated to be more or less than 0.6 µg BaP/kg.

RESULTS

Samples A-1 and A-2 (real concentration about 0.1 µg BaP/kg) were analyzed by 7 laboratories each. All laboratories correctly found that the concentration was <0.6 µg BaP/kg sample. Since A-1 and A-2 were identical samples, 13 correct answers were obtained (Lab. 6 did not contribute to sample A-2).

Samples A-3 and A-4 (real concentration about 0.4 µg BaP/kg) were analyzed by 15 laboratories. Due to the fact that the real BaP-concentration was close to the decision border of 0.6 µg BaP/kg and to the fact that the background fluorescence tend to lead to overestimations of the concentration, 6 out of 30 answers (no data for A-4 in case of lab 6 and lab 12; duplicate determination in case of lab 7; A-3 and A-4 were identical samples) were wrong. All results obtained are summarized in Table 1.

REFERENCES

1. Directive on smoked meat, 4th July 1978, Bundesgesetzblatt I, p. 1003.

2. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG of the Federal Republic of Germany. Untersuchung von Lebensmitteln. Bestimmung von Benzo(a)pyren in geräucherten Fleischerzeugnissen (Screening-Verahren) Methode I, Mai 1983, L 07.00-26.