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RECOMMENDED METHOD FOR THE GAS CHROMATOGRAPHIC PROFILE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED FOOD

Prepared for publication by

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Recommended method for the gas chromatographic profile analysis of polycyclic aromatic hydrocarbons in smoked food

Abstract - Results are reported of a collaborative study on the determination of polycyclic aromatic hydrocarbons (PAH) including benz(a)anthracene, chrysene, benzofluoranthenes (b+j+k), benzo(a)pyrene, benzo(e)pyrene, and indeno(1,2,3-cd)pyrene in smoked meat. After calibration of the instrument with a standard solution, four samples of smoked meat (two of each were identical samples, respectively) were analysed. Variation coefficients for the above PAH were found to be 19.5-57.5 % with the exception of indeno-(1,2,3-cd)pyrene for which a coefficient of 105.5- 126.9 % was found due to an external impurity which interferes with this PAH in GC.

INTRODUCTION

Although in many environmental studies only benzo(a)pyrene has been regarded as a carcinogenic representative of PAH, it is well-known that other individuals of this class considerably contribute to the total carcinogenicity of various environmental matter (1, 2). As a consequence, a profile analysis is required which allows the simultaneous determination of as many as possible PAH. Due to legislative regulations which restrict the concentration of six selected PAH to 200 or 250 ng/L water, a thin-layer chromatographic determination method has been developed which is widely used for water control (3). Since, however, resolution for additional PAH is poor with this method, for more sophisticated problems gas chromatography is applied. A previously described GC method (4) has been used in an internal collaborative study including 7 laboratories.

SCOPE AND FIELD OF APPLICATION

The method specifies a procedure for the gas chromatographic profile analysis of PAH (including benz(a)anthracene, chrysene, benzofluoranthenes (b+j+k), benzo(a)pyrene, benzo(e)pyrene and indeno(1,2,3-cd)pyrene) in smoked meat. It also can be applied to other materials and allows, moreover, the determination of other PAH as well. The enrichment procedure specifically separates PAH which are gas-chromatographically analysed and quantified by comparison with internal standards. The method has a limit of detection of about 0.3 ng PAH.

PRINCIPLE

The sample is spiked with two standards (indeno(1,2,3-cd)fluoranthene and benzo(b)chrycene), and 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 with methanol/water (7+3). In case of TCFE extraction, this is removed by distillation and the residue is then dissolved in 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 the PAH extracted with cyclohexane. The cyclohexane phase is washed with water and filtered through silica. After cautious evaporation to near dryness the residue is dissolved in isopropanol and separated by chromatography on Sephadex LH 20 by elution with isopropanol into PAH with 2 and 3 rings and a fraction containing PAH with more than 3 rings. After evaporation of the solvent to near dryness the latter fraction is redissolved in toluene (10-20 µL) and analysed by GC using capillary columns.

A schematic representation of the procedure is given in Figure 1.

MATERIAL

All solvents used (cyclohexane, methanol, dimethylformamide, isopropanol (propanol-2), acetone) were of analytical grade and redistilled in carefully cleaned glass vessels before use. Before analyzing a sample a blank test following the entire procedure should be carried out.

For column chromatography silica 60 (particle size 0.063-0.200 mm ≙ 70-230 mesh ASTM) and Sephadex LH 20 (Pharmacia Fine Chemicals, S-75104 Uppsala 1, Sweden) were used.

The following reference PAH were obtained in a better than 99.0 % purity from the Commission of the European Community, Community Bureau of Reference - BCR, Rue de la Loi 200, B-1049 Brussels, Belgium: fluoranthene, pyrene, benzo(c)phenanthrene, benzo(ghi)fluoranthene, benz-(a)anthracene, chrysene, triphenylene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)-fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, benzo(ghi)-perylene, anthanthrene, coronene as well as the internal standard indeno(1,2,3- cd)fluoranthene and benzo(b)chrysene.

A reference solution for the calibration of the GC instrument and for the determination of the GC retention times contained the following PAH: benz(a)anthracene (5.20 ng/µL); chrysene

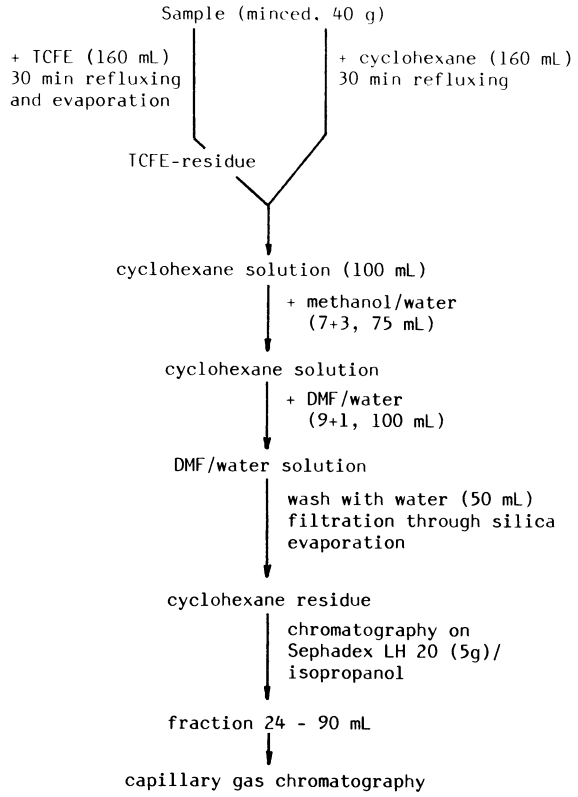

Fig. 1. Schematic representation of the procedure
TCFE = 1,1,2-trichlorotrifluoroethane
DMF = N,N-dimethylformamide

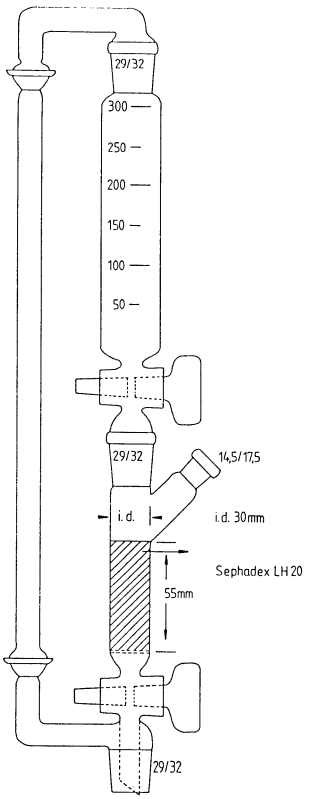

Fig. 2. Glass column for chromatography on Sephadex LH 20 (closed system) avoiding contact to ambient air.

(3.75 ng/µL); benzo(b)fluoranthene (1.82 ng/µL); benzo(e)pyrene (6.57 ng/µL); benzo(a)pyrene (3.25 ng/µL), indeno(1,2,3-cd)pyrene (2.97 ng/µL). The standard solutions contained indeno-(1,2,3-cd)fluoranthene (8.20 ng/µL) and benzo(b)chrysene (14.46 ng/µL), respectively.

Four samples of minced ham (40 g each) (A-1; A-2; B-1 and B-2) were distributed. A-1/A-2 as well as B-1/B-2 were identical samples. The A-samples were spiked with PAH, whereas the B-samples were unspiked commercial samples.

APPARATUS

For chromatography on Sephadex LH 20 a closed column (Figure 2) is used.

Gas chromatograph: Perkin-Elmer & Co., model Sigma 2B fitted with a flame ionization detector or equivalent instrument. Electronic integrator Spectra Physics SP 4100-02 or equivalent instrument.

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, covered with TCFE (160 mL) (TCFE can be replaced by 160 ml cyclohexane) and spiked with two internal standards (indeno(1,2,3-cd)fluoranthene, 200 ng, and benzo(b)chrysene, 200 ng).

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, filtered and evaporated to near dryness using a rotatory evaporator (reduced pressure, water bath temperature 35 ^{o}C). It is then redissolved in 100 mL cyclohexane.

1.2.2 In case of cyclohexane extraction the solution is cooled to room temperature, filtered and evaporated to 100 mL.

1.3 The cyclohexane solution (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. The methanol-water phase is discarded.

2. Liquid–liquid partition

2.1 N,N-dimethylformamide (90 mL) and water (10 mL) are added to the cyclohexane solution (of 1.3) and the mixture is shaken (about 5 min) then allowed to separate and the upper layer (cyclohexane) is 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 ^{o}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 ^{o}C).

3.5 The residue is dissolved in isopropanol (about 1 mL).

4. Chromatography on Sephadex LH 20

4.1 The column is prepared as follows:
30 mL isopropanol are added to 5 g Sephadex LH 20 and equilibrated for some hours. The slurry is added to the chromatographic column and the solvent run until liquid level coincides with the upper surface of the packing material.

4.2 The solution is transferred from 3.5 to the column and the flask rinsed with 1 mL isopropanol and also transferred to the column.

4.3 The extract and rinsing are allowed to run down into the column, stopping when solvent coincides with the surface of the packing material.

4.4 The column is eluted with 24 mL isopropanol and this fraction is discarded.

4.5 The column is further eluted with 70 mL isopropanol and the eluate collected in a 100 mL-round-bottomed flask (this fraction contains all PAH with more than 3 rings).

4.6 This fraction is evaporated to near dryness, using a rotatory evaporator.

5. Preparation of the fraction for gas chromatography

5.1 About 0.5 ml acetone are added to the residue (of 4.6) and transferred quantitatively to a concentration tube (about 5-10 mL volume).

5.2 The flask of 4.6 is rinsed again with 1 mL acetone and added to the same concentration tube.

5.3 Acetone is evaporated to near dryness, using a rotatory evaporator, taking care to avoid splashing to the walls of the concentration tube.

5.4 Using a rotatory evaporator, it is necessary to rinse the wall of the tube with acetone (0.5 mL) again. Repeat 5.3.

5.5 10 µL toluene are added to the residue and retained for capillary gas chromatography.

6. Glass-capillary-gas-chromatography

6.1 Gas chromatographic conditions:
Column: 0.3 mm x 25 m, coated with poly-dimethylsiloxane (e.g. CP sil 5, SE 30, OV 101 (or fused silica columns - poly dimethylsiloxane, chemical bound), carrier gas: helium 2 mL/min, injection port temperature: 250 ^{o}C, detector block temperature: 270 ^{o}C. To control the response factors of the FID, a reference mixture containing several PAH is used. The PAH-masses (weights) and the recorded areas of the FID-signal result in a constant quotient.

6.2 Procedure:
1 µL of the PAH-reference mixture containing original substances is injected to control the ratio of the masses of each PAH to the area of the FID-signal and the retention time of this PAH. The PAH-mixture, dissolved in toluene from 5.5, is injected without splitting at 110 ^{o}C using a 10 µL syringe, about 5 µL of the 10 µL solution may be injected. After 5 min the split is opened and the column is heated to 160 ^{o}C by 30 ^{o}C/min, followed by a temperature program of 1.5 ^{o}C/min to the final temperature of 270 ^{o}C. In Fig. 3 a gas chromatogram is presented, recorded under these conditions.

6.3 Short-time-procedure:
Alternatively to the procedure described in 6.1 and 6.2, a short-time program can be used for the determination of benzo(a)pyrene exclusively.
Conditions: Column 0.3 mm x 10 m, poly-dimethylsiloxane CP sil5, helium 25 cm/sec; injection port temperature: 260 ^{o}C.
Inject 5 µL of the sample (dissolved in 10 µL toluene, see under 5.5) at 110 ^{o}C using 10 µL syringe without splitting. After 4 min the split is opened and the column heated to 230 ^{o}C by 30 ^{o}C/min, followed by 5 ^{o}C/min to the final temperature of 280 ^{o}C.

TABLE 1. Results of the analysis of PAH spiked meat samples (A-1 and A-2) (ng/40 g)

Laboratory	1		2		3		4		5		6		7
Sample	A-1	A-2	A-1	A-2	A-1	A-2	A-1	A-2	A-1	A-2	A-1	A-2	A-1
INF (added)	259.5		200		191		198		230		300		200
BbC (added)	261.0		200		190		192	181	228		345.2		200
BaA (46.8)**	32.5	28.3	11	153	38	29	44	49	20.9	16.1	50.4	45.7	136.8
CHR (33.8)**	33.9	29.8	14	30	48	40	35	36	44.5	56.2	38.6	34.2	87.2*
BF (16.4)**	23.4	21.8	7	12	25	20	19	15	18.9	16.5	16.5	17.4	153.6*
BeP (59.1)**	35.3	51.5	16	30	51	43	56	56	113.1*	59.3	65.6	63.5	26.8
BaP (53.4)**	43.3	43.2	16*	49	40	37	47	48	63.5	31.9	48.5	55.3	35.2
INP (13.4)**	21.7	19.3	119	182*	-	-	221*	35	9.5	123.9	12.9	13.4	32.0
BbC (found)	330.2	313.7	-	95	210	210	174*	188	210.2	240.8	351	365	-

* values rejected as outliers
** amount for spiking samples
INF = indeno(1,2,3-cd)fluoranthene
BF = benzofluoranthenes (b+j+k)
BbC = benzo(b)chrysene
BeP = benzo(e)pyrene
BaA = benz(a)anthracene
BaP = benzo(a)pyrene
CHR = chrysene
INP = indeno(1,2,3-cd)pyrene

TABLE 2. Results of the analysis of natural meat samples (B-1 and B-2) (ng/40 g)

Laboratory	1		2		3		4		5		6	
Sample	B-1	B-2	B-1	B-2	B-1	B-2	B-1	B-2	B-1	B-2	B-1	B-2
INF (added)	259.5		200		191		198	195	255	262	449.4	
BbC (added)	261.0		200		190		181		217	254	517.1	
BaA	60.5	51.3	60	61	41	32	93	91	35	36.1	96.8	114.5
CHR	110.5	98.6	121	123	84	60	138	160	162	155.9	172.4	178.5
BF	28.1	45.4	47	55	27	25	17	16	25	36.3	23.0	24.5
BeP	21.5	14.6	134*	93*	19	16	34	34	32	27.5	32.3	31.5
BaP	32.7	21.2	109*	105*	19	18	26	32	14	22.7	45.4	45.4
INP	-	-	182*	178*	-	-	25	49	230*	20.0	12.0	10.8
BbC (Found)	323.8	303.1	106	109	199	207	198	163	272	245	490	495

*values rejected as outliers for abbreviations see Table 1

TABLE 3. Means and coefficients of variation before and after elimination of outliers for various PAH of A- and B-samples.

	Sample A				Sample B			
	$\bar{x}_{13}$*	V_{13}(%)*	$\bar{x}$**	V(%)**	$\bar{x}_{12}$*	V_{12}(%)*	$\bar{x}$**	V(%)**
BaA	50.1	87.5	50.1	57.5	64.4	43.4	64.4	43.4
CHR	40.6	42.5	36.7	28.6	130.3	28.6	130.3	28.6
BF	28.1	134.9	17.7	27.9	30.8	40.3	30.8	40.3
BeP	51.3	46.7	46.1	34.4	40.8	87.7	26.2	29.4
BaP	42.9	27.2	45.2	19.5	40.9	79.5	27.6	40.0
INP	60.7	123.0	42.0	105.5	58.9	144.6	13.0	126.9

*means and coefficients of variation of 13 and 12 values, respectively
** means and coefficients of variation after elimination of outliers

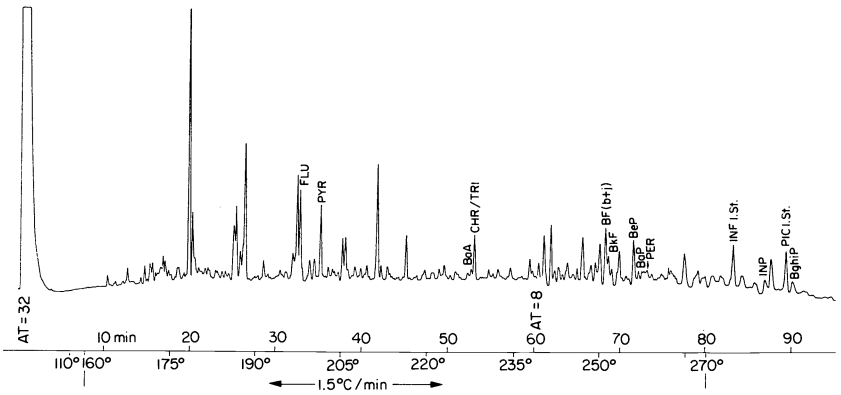

Fig. 3. PAH-profile of smoked ham (retention time of benzo(a)pyrene: about 70 min). Conditions of glass capillary gas chromatography see 6. The height of indeno(1,2,3-cd)-fluoranthene signal corresponds to 18.3 ng, that of benzo(b)chrysene to 19.0 ng (AT=8), FID.

7. Characterization and quantification of the PAH-signals

Use of two internal standards (indeno(1,2,3-cd)fluoranthene and benzo(b)chrysene) allows the characterization of various PAH by their relative retention times.

Results are calculated by comparing the electronically recorded areas of the FID-signals of each PAH with the areas of the two internal standards. Indeno(1,2,3-cd)fluoranthene is more stable than benzo(b)chrysene, and hence the quality of the glass capillary column can be checked by the ratio BbC/INF.

It is also possible to compare the signal height of each PAH from the sample to that of the corresponding PAH from the reference solution. In case of satisfactory repeatability of the temperature program the quantification of the PAH from a sample can be achieved without electronical intergration.

8. Control for completeness of the extraction (see 1.1-1.4)

To check the completeness of the PAH-extraction, the meat sample extracted with 1,1,2-trichlorotrifluorethane is saponified by methanolic alkaline (2N, methanol + water, 9+1) after addition of another internal standard (e.g. dibenz(a,h)anthracene) for several hours. After saponification, the solution (methanol + water, 9+1) is extracted by an equal volume of cyclohexane. The clean-up of the cyclohexane phase follows the above procedure using gas chromatographic determination. This check should be carried out occasionally.

The residual BaP-content of the sample after extraction with TCFE is about 6 % BaP of the total amount after a single extraction.

RESULTS

The participating laboratories were requested to compare the areas of the FID-signals from the 8 PAH and that from the second internal standard (benzo(b)chrysene) with the response of the first internal standard (indeno(1,2,3-cd)fluoranthene) the amount of which to add to the samples were at free choice (about 200 ng).

Regarding the fact that A-1/A-2 and B-1/B-2 each were duplicate samples, a total of 13 results were obtained for the A-samples (lab 7 analyzed only A-1) and 12 results for the B-samples. Due to methodological difficulties (GC interference with a contaminant) some laboratories did not give values for indeno(1,2,3-cd)pyrene and those who delivered data for it commented on the limited reliability of these data. As a consequence, very high coefficients of variation for the determination of this PAH were obtained (123.0-144.6 %). According to statistical calculations some data had to be rejected as outliers. They are marked by * in Tables 1 and 2 which summarize the results obtained. The final coefficients of variation (Table 3) were 19.5- 57.5 for all PAH with the exception of indeno(1,2,3-cd)pyrene for which still a coefficient of variation of 105.5-126.9 % was found. Accordingly, the determination of this PAH is not yet satisfactory.

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