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Recommended Method for the

GAS CHROMATOGRAPHIC PROFILE ANALYSIS OF BASIC N-CONTAINING AROMATIC COMPONENTS (AZAARENES) IN HIGH PROTEIN FOODS

Prepared for publication by

GERNOT GRIMMER

Biochemisches Institut für Umweltcarcinogene
2070 Ahrensburg, FRG

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RECOMMENDED METHOD FOR THE GAS CHROMATOGRAPHIC PROFILE ANALYSIS OF BASIC N-CONTAINING AROMATIC COMPONENTS (AZAARENES) IN HIGH PROTEIN FOODS

Abstract - Results are reported of a collaborative study on the determination of basic nitrogen-containing aromatic compounds (azaarenes) in high protein foods. To this end reference solutions and spiked ham samples were analysed. From some 20 samples coefficients of variation between 4.0-13.6% were found in case of the reference solution and 10.4-25.4% in case of the spiked sample.

Since several nitrogen-containing polycyclic aromatic compounds have been shown to possess carcinogenic and mutagenic effects, it was decided by the Food Section of the IUPAC that a gas chromatographic method using capillary columns should be developed for the determination of the compounds with the participation of laboratories in several countries (listed at the end).

SCOPE AND FIELD OF APPLICATION

This method specifies a procedure for determination of basic nitrogen-containing polycyclic aromatic compounds such as azaarenes in a wide range of products such as fresh, broiled, smoked, and grilled meat, sausages, ham, fish, poultry etc.

The enrichment procedure yields basic compounds only such as acridine,benzacridines, dibenz-acridines, aza-pyrenes, and related aza-arenes and methyl derivatives thereof, but not neutral compounds such as carbazoles etc.

This method has a limit of detection ranging from 0.1 ng to 0.4 ng [e.g. 0.1 ng for benz(c)acridine; 0.2 ng for 8,10-dimethylbenz(a)acridine; 0.4 ng for dibenzacridines] .

PRINCIPLE

The sample is minced by a mincing-machine (e.g. mechanical blender) to a homogeneous squash in order to avoid the saponification procedure and extracted by 1,1,2-trichlorotrifluorethane after adding the internal standard. The residue of this extract is dissolved in cyclohexane and extracted with a mixture of N,N-dimethylformamide (DMF) and water (9+1).

The DMF-water-phase is diluted with water (twice the volume of the DMF-water-phase) and extracted with cyclohexane (3 times). After washing and evaporation, the residue is dissolved by cyclohexane again and shaked with H_2SO_4 to collect the basic compounds. After neutralisation, the solution is extracted by 1,1,2-trichlorotrifluoethane and purified by column chromatography on Sephadex LH 20, using a closed system to avoid air pollution. N-PAC are then analysed by glass capillary gas chromatography.

Results are calculated by comparing the areas of the FID-signals of the compounds to those of the internal standard [e.g. 10-aza-benzo(a)pyrene or 8,10-dimethylbenz(a)acridine] .

Schematic presentation is shown in Fig.1. The method is submitted for publication to J.A.O.A.C.

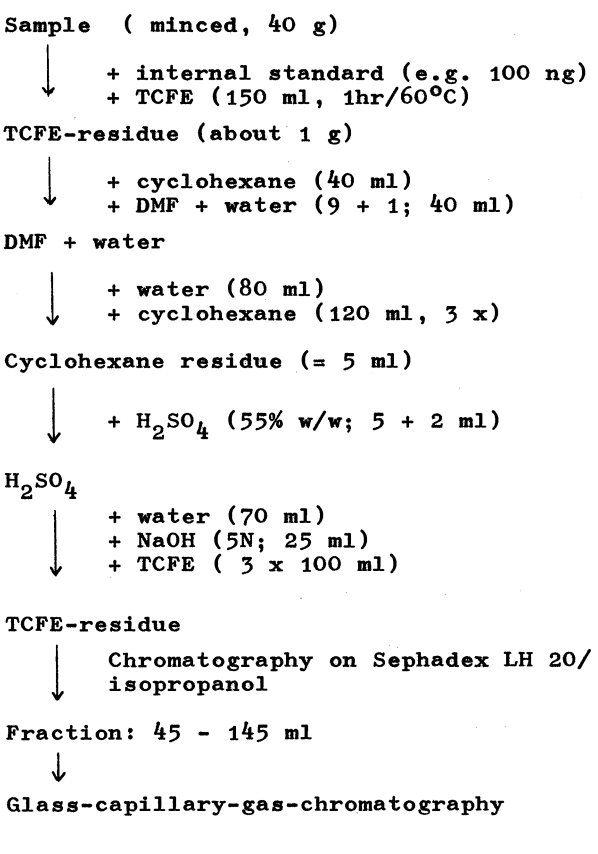

Fig.1 Schematic representation of procedure
(TCFE = 1,1,2-Trichlorotrifluoroethane;
DMF = N,N-Dimethylformamide)

MATERIALS

(1) Reference solution: a mixture of aza-arenes, dissolved in toluene + pyridine (99 + 1)

benz(c)acridine	BcAC	dibenz(c,h)acridine	DBchAc
8,10-dimethylbenz(a)acridine	8,10-D	dibenz(a,h)acridine	DBahAC
10-azabenzo(a)pyrene		dibenz(a,j)acridine	DBajAC

The concentration of these compounds was in the range of 10-40 ng/µl (see Table 1).

(2) Sample 1 and 2: minced ham for 2 analyses (2 x 40 g), no difference between the two samples. The samples were spiked with the following azaarenes: benz(c)acridine, dibenz(c,h)acridine, dibenz(a,h)acridine, and dibenz(a,j)-acridine in the range of some ng/g. Apart from these azaarenes, the samples contained some 'native' N-containing aromatic compounds.

RESULTS

(1) Analysis of the reference solution

The reference solution contains the internal standard 10-azabenzo(a)pyrene and 5 other heterocyclic compounds. The composition of this mixture is shown in Table 1. Participating laboratories were requested to compare the areas of the 5 compounds with the area of the FID-signal of the internal standard which corresponds to 24.6 ng/µl 10-azabenzo(a)pyrene.

Some 20 analyses were received but in some cases the values had to be cancelled for statistical reasons. The cancelled values are in brackets, e.g. in the first, second, and in the third line under the head of Table 1. The last two lines represent the average of all values except the cancelled ones (in brackets all values), as well as the coefficient of variation.

The standard deviation is satisfying. This is indicated by the coefficient of variation, ranging from 4.0 -13.6%.

Table 1. Results of the analysis of the reference solution

Internal standard: 10-azabenzo(a)pyrene (24.6 ng/µl)

Acridines: benz(c)acridine (BcAC)
8,10-dibenz(a)acridine (8,10-D)
dibenz(c,h)acridine (DBchAC)
dibenz(a,h)acridine (DBahAC)
dibenz(a,j)acridine (DBajAC)

	BcAC	8,10-D	DBchAC	DBahAC	DBajAC
Theoretical (ng)	11.6	23.1	18.2	21.1	41.0
Lab. 1	15.7	22.9	(13.3)	(13.7)	(28.8)
	17.4	24.6	(11.6)	(11.9)	(23.1)
	16.2	23.0	(16.3)	(13.9)	(31.2)
2	10.4	(19.4)	17.0	18.3	38.5
	11.0	(20.3)	16.9	18.8	39.0
	10.9	(20.0)	16.8	18.4	37.8
3	12.0	22.8	18.3	21.2	40.8
	11.8	22.8	18.3	20.8	41.0
	12.6	22.7	21.2	21.7	40.3
4	13.2	24.0	18.2	19.5	38.5
	13.2	23.6	18.6	20.1	40.4
	12.9	23.1	18.5	19.9	40.2
5	13.0	24.1	19.7	20.5	40.9
	13.9	25.2	19.5	20.2	42.0
	14.4	25.4	18.8	20.1	41.1
6	12.1	22.7	19.6	21.1	43.8
	11.9	22.3	19.2	20.7	43.0
	11.7	22.9	19.3	21.0	42.5
7 (3)	13.0	24.6	19.7	21.3	44.9
Average (ng)	13.0	23.7	18.8	20.3	41.4
	(13.1)	(23.1)	(18.0)	(19.2)	(39.3)
Variation coefficient (%)	13.6	4.0	5.5	5.2	5.8

(2) Analysis of the spiked samples

The results of the analysis of the spiked samples (40 g) are demonstrated in Table 2 ; some analyses were cancelled for statistical reasons.

Some 20 analyses were received from 10 laboratories. The first and second line originates from the same laboratory, the third line gives the average value of these 2 analyses.

The coefficients of variation range from 10.4% to 25.4%. This is a good result if one keeps in mind that the sample of 40 g contains only about 40 ng DBchAC or 100 ng of BcAC.

Table 2. Results of the analysis of spiked meat samples

Internal standard: 10-azabenzo(a)pyrene

Sample spiked with (µg/kg)	BcAC 2.73	DBchAC 1.23	DBahAC 3.13	DBajAC 6.53
Lab. 1	3.2	1.2	3.2	6.0
	2.7	0.6	2.4	6.6
$\bar{x}$	2.95	0.9	2.8	6.3
2	2.5	1.5	3.0	5.0
	2.1	1.3	3.1	5.5
$\bar{x}$	2.3	1.4	3.05	5.25
3	(1.5)	2.5	-	3.3
	(0.6)	0.9	-	2.9
$\bar{x}$	(1.05)	1.7	-	3.1
4	2.9	1.2	3.8	4.1
	3.0	1.3	4.1	4.9
$\bar{x}$	2.95	1.25	3.95	4.5
5	2.4	1.2	3.0	4.4
	2.8	1.2	3.3	9.9
$\bar{x}$	2.6	1.2	3.15	7.15
6	2.7	1.0	2.7	5.9
	3.2	0.9	2.3	5.6
$\bar{x}$	2.95	0.95	2.5	5.75
7	2.6	1.2	3.1	3.8
	2.4	1.1	3.2	5.3
$\bar{x}$	2.5	1.15	3.15	4.55
Average (ppm)	2.7	1.2	3.1	5.2
Variation coefficient (%)	10.4	22.3	10.7	25.4

List of the Participants

M.Ozaki, I.Ono and T.Kato
Ajinomoto Co.,Inc.
Central Research Laboratories
1-1 Suzuki-cho, Kawasaki-ku
Kawasaki 210, Japan

G.R.Scott
Government Analyst
Dept.of Scientific and Industrial Research
P.O.Box 2112
Christchurch, New Zealand

Adele Candeli
Universita, Istituto di Igniene
Facolta Scienze
Via del Giochetto
I-06100 Perugia, Italy

Georg Becher
Sentralinstitutt for Industriell Forskning
Forskningsv.1
P.B.350 Blindern
N-Oslo 3, Norway

Zelta Penton
Varian Instruments Group, Walnut Creek Division
2700 Mitchell Drive
P.O.Box 9016, Walnut Creek
California 94598, U.S.A.

H.A.M.G.Vaessen
Abt.Allgemeine Chemische Analyse
Rijksinstituut voor de Volksgezondheid
Antonie van Leeuwenhoek lann 9
Postbus 1
NL-3720 BA Bilthoven

W.Karcher
Abt.Werkstoffe
Joint Research Centre
Petten Establishment
Postbus 2
NL-1755 ZG Petten

Gernot Grimmer
Biochemical Institute for Environmental Carcinogens
Sieker Landstraße 19
D-2070 Ahrensburg