Quantification of 15+1 EU Priority PAH in representative German smoked meat products using a Fast GC/HRMS Method.

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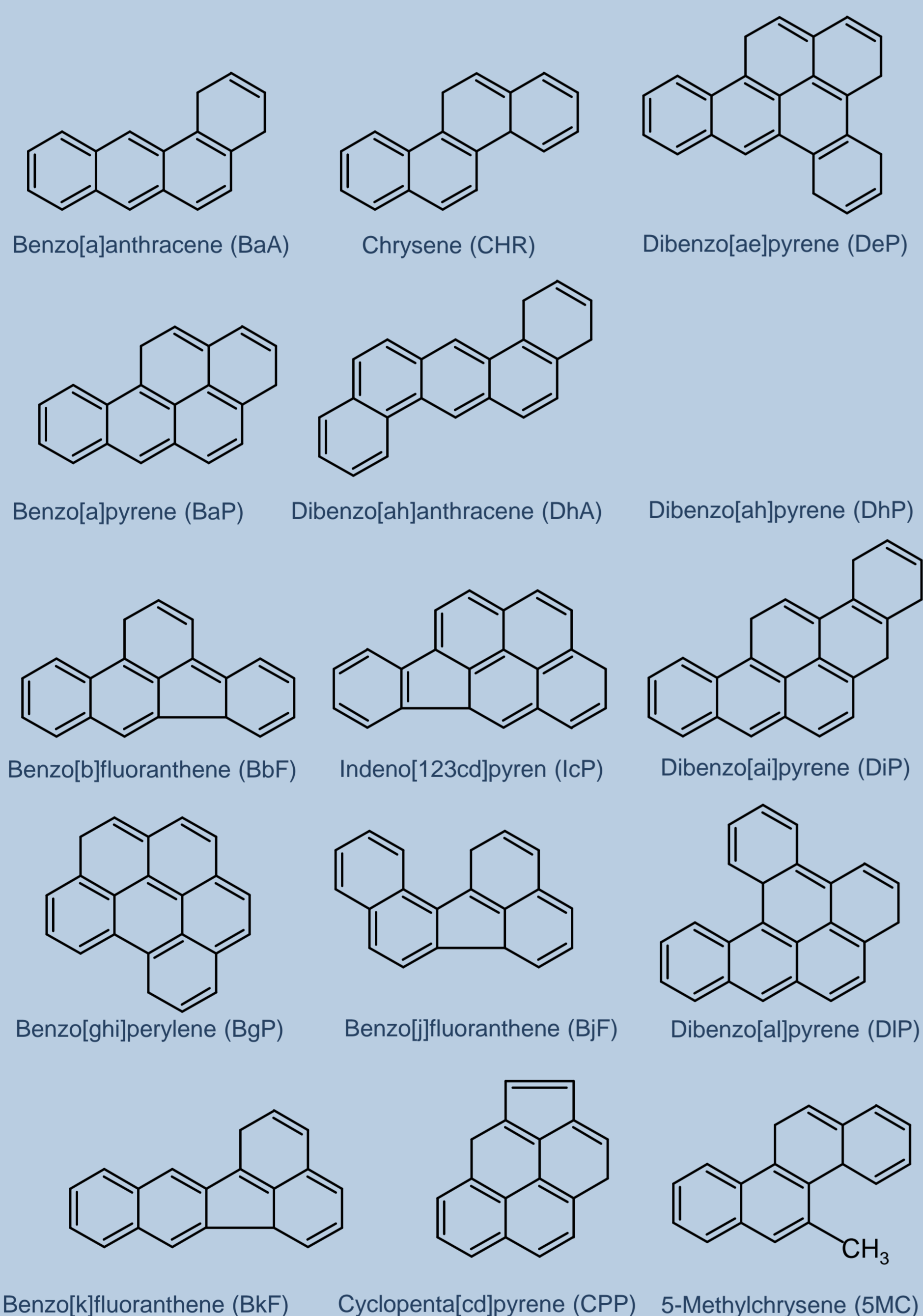
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Overview

Purpose: For the analysis of the 15 polycyclic aromatic hydrocarbons (PAH) classified as priority from the Scientific Committee on Food (SCF) and benzo[c]fluorene assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA) in food, a sensitive analytical method is necessary.

Methods: A Fast-GC/HRMS method with a runtime of only 25 minutes using a TR-50ms column (10m x 0.1mm x 0.1μm) was developed.

Results: 113 representative samples of commercial smoked German meat products were tested. The median of benzo[a]pyrene content was 0.03 μg/kg and therefore greater than a factor of 100 below the maximum level of 5 μg/kg. The determined LODs and LOQs were in the range of 0.003 - 0.01 μg/kg and 0.009 – 0.03 μg/kg, respectively.

Introduction

In the European Union, a maximum level of 5 μg/kg benzo[a]pyrene (BaP) in smoked meats and smoked meat products exists [1]. Also, the Scientific Committee on Food (SCF) has recommended the member states of the European Union to analyse the contents of 15 PAH compounds, which are classified as priority (15 SCF-PAH), and to check the suitability of BaP as a marker for the occurrence and impact of carcinogenic PAH in food [2]. Additionally, the European Food Safety Authority (EFSA) recommends the analysis of benzo[c]Xuorene (BcL), assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA) [3].

Benzo[a]anthracene (BaA)   Chrysene (CHR)   Dibenzo[ae]pyrene (DeP)

Benzo[a]pyrene (BaP)   Dibenzo[ah]anthracene (DhA)   Dibenzo[ah]pyrene (DhP)

Benzo[b]fluorene (BbF)   Indeno[123cd]pyren (IcP)   Dibenzo[ai]pyrene (DiP)

Benzo[ghi]perylene (BgP)   Benzo[j]fluoranthene (BjF)   Dibenzo[al]pyrene (DIP)

Benzo[k]fluoranthene (BkF)   Cyclopenta[cd]pyrene (CPP)   5-Methylchrysene (5MC)

FIGURE 1. 15 PAH priority compounds classified by the European Commission regulation.

Benzo[c]fluorene

**FIGURE 1b. Additional according to JECFA**
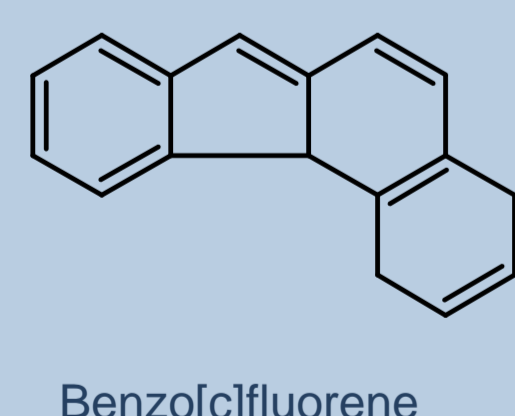
The 15+1 EU priority PAH that are recommended to be analyzed include 15 SCF-PAH and JECFA PAH are shown in Fig. 1a and 1b. "PAH4" is the sum content of BaP, CHR, BaA and BbF.

Methods

Sample preparation

Starting from 5 g ham or 3 g other meat products the homogenized samples were spiked with isotope-labelled (^{13}C and ^{2}H) and fluorinated PAH standards. The extraction was performed with an accelerated solvent extraction ASE 200 (Dionex), followed by a gel permeation chromatograpy and solid phase extraction with a modified ASPEC Xli (Gilson). The dried SPE isooctane extract was spiked with the PAH- Recovery standard mixture and concentrated to a final volume of 50μl.
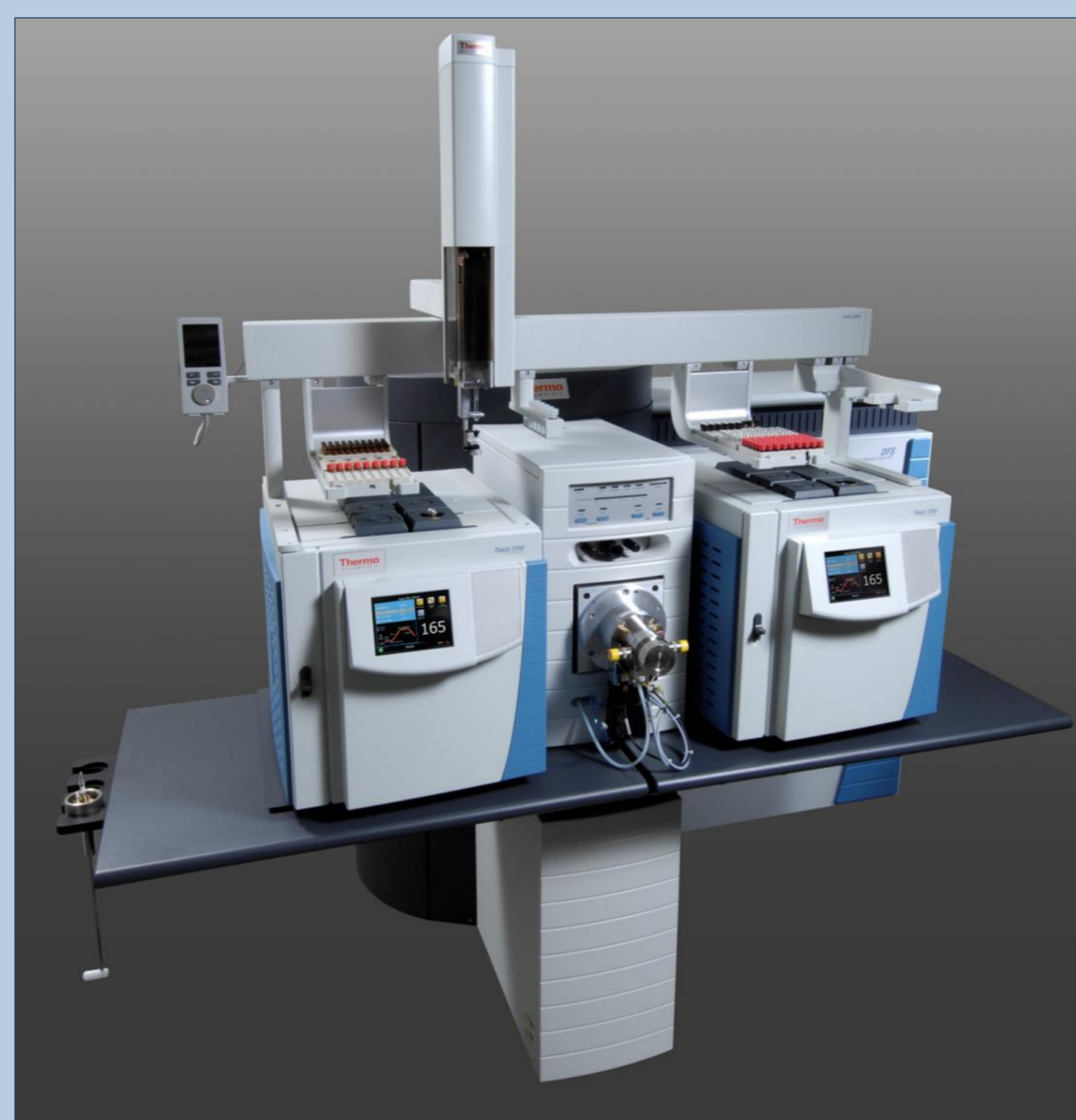

FIGURE 2. Thermo Scientific DFS High Resolution Magnetic Sector MS System.

Mass Spectrometry

All experiments were carried out on a Thermo Scientific DFS^{TM} High resolution mass spectrometer. A multi window selected ion monitoring (SIM) method containing all optimised analyte ions was set up; including the usage of suitable reference masses. A mass resolution of 8,000 (10 % valley) was employed for ultimate selectivity.

Gas Chromatography

Extracts and standards were injected in splitless mode via split/splitless injector on a Thermo TR-50MS column (10m x 0.1mm x 0.1μm).
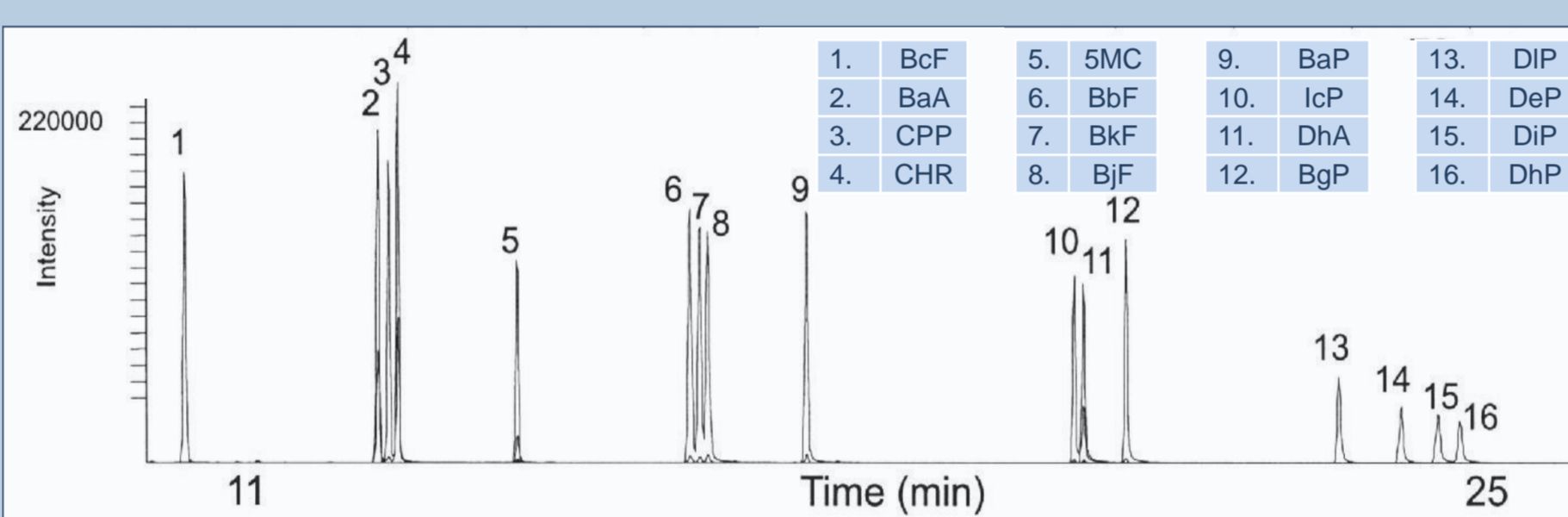
FIGURE 2. Eluting order of 15+1 priority PAHs using a Fast GC methode.

A fast GC method was developed with a approx. 25 min retention Time maintaining chromatographic resolution for all 15+1 PAHs.

Table 1. GC and MS Parameters

GC parameters	
Carrier gas flow rate	0.6 mL/min
Oven temp. Program	140 °C (1min)
	10.0 °C/min – 240 °C
	5 °C/min – 270 °C
	30.0 °C/min – 280 °C
	4.0 °C/min – 290 °C
	30.0 °C/min – 315 °C
	3.0 °C/min – 330 °C
Transfer line temp.	300 °C
SSL Injector	320 °C
MS Parameter	
Ionization Mode	EI positive Ion
Electron Energy	45 eV
Ion source temperature	280 °C
Resolution (10% valley)	8000
AS Parameter	
Injection volume	1.5 µL (spitless)

Results

A total of 113 samples of smoked meat products (raw sausages (N = 25), raw ham (N = 23), cooked ham (N = 17), Frankfurter-type sausages (N = 23) and liver sausages (N = 25)) were analyzed.

As shown in Table 2, the standard deviations between analysed contents and spiked contents were in the range of –16 to +13% for all PAH compounds with the exception of DiP (–32%). Consequently, the standard deviations of all PAH compounds with the exception of DiP were in the range of the target standard deviation of 22% used in the interlaboratory comparison [4]. Considering the repeatability, the analysis of ten samples including the complete sample preparation) resulted in standard deviations in the range of 4–16%. With the exception of BgP, DiP and DhP, the standard deviations were below 10%. The analysis of meat reference material with spiked concentrations in the range of 4.1 to 9.9 mg/kg resulted in recoveries from 68 to 113% shown in Table 2.

Table 2. Spiked contents [4], analysed contents (μg/kg) and recovery (%) of PAH compounds in meat reference materials

Component	Spiked [μg/kg]	Analyzed (N=10) [μg/kg]	Recovery [%]
5-Methylchrysene	6.2	5.2 ± 0.4	84
Benzo[a]anthracene	5.9	5.3 ± 0.3	90
Benzo[a]pyrene	5.3	5.2 ± 0.3	98
Benzo[b]Xuoranthene	4.1	3.8 ± 0.2	93
Benzo[c]Xuorene	3.9	4.1 ± 0.2	105
Benzo[g,h,i]perylene	4.7	4.1 ± 0.5	87
Benzo[j]Xuoranthene	9.2	8.3 ± 0.4	90
Benzo[k]Xuoranthene	5.2	4.8 ± 0.2	92
Chrysene	6.4	5.5 ± 0.3	86
Cyclopenta[c,d]pyrene	6	5.9 ± 0.5	98
Dibenzo[a,e]pyrene	7.1	8.0 ± 0.4	113
Dibenzo[a,h]anthracene	7.7	7.0 ± 0.3	91
Dibenzo[a,h]pyrene	9.9	8.5 ± 1.4	86
Dibenzo[a,i]pyrene	5.6	3.8 ± 0.4	68
Dibenzo[a,l]pyrene	7.7	7.3 ± 0.5	95
Indeno[1,2,3-cd]pyrene	5.2	4.8 ± 0.4	92

The different PAH compounds were detectable with different limits of detection (LOD) and limits of quantification (LOQ). The LOD, which is defined as the lowest concentration of an analyte detectable in a sample (signal-to-noise ratio = 3:1), and the LOQ, which is defined as the lowest content of the analyte that can be measured with reasonable statistical certainty (signal-to noise ratio = 9:1) for the different PAH compounds are shown in Table 3.

Table 3. Limit of detection (LOD) and limit of quantification (LOQ) of the individual PAH in smoked meat products

Component	LOD (μg/kg)	LOQ (μg/kg)
BcL, BaA, CHR, CPP, 5MC, BbF, BkF, BjF, BaP, BgP	0.003	0.009
IcP, DhA, DIP, DeP, DiP, DhP	0.01	0.03
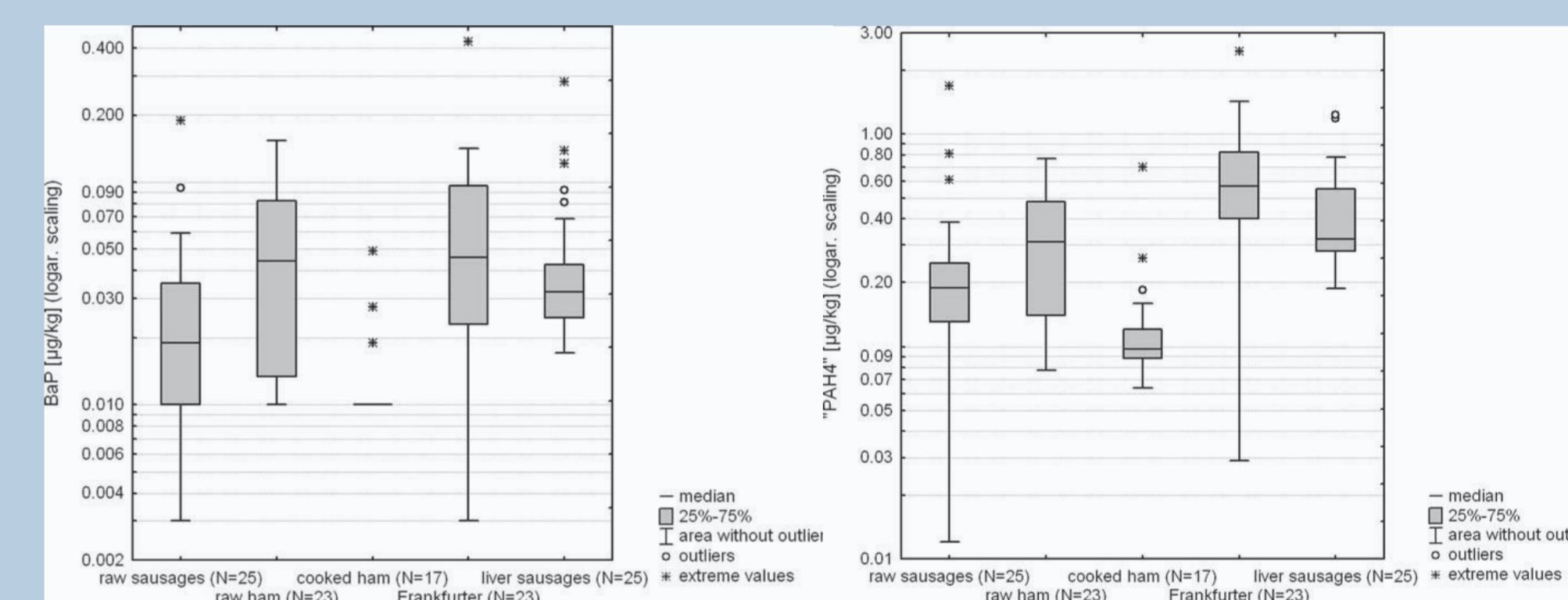
FIGURE 5. Box plots of the BaP (left) and „PAH4" CHR, BaA, BbF and BaP (right) in different types of meat products (N=113)

The correlation coefficient (R) between benzo[a]pyrene and the sum of the 15+1 EU priority PAH was 0.90. For the sum content ("PAH4") a correlation coefficient of R=0.99 was calculated.

Conclusion

The required analytical parameters limit of detection (LOD) and limit of quantification (LOQ) for this type of low level samples can be achieved with a high resolution sector field instrument for all individual 15+1 PAH.

- A fast GC method was successful developed with an retention time of approx. 25 min.

References

1. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union L 364:5 - 24

2. Commission recommendation of 4 February 2005 on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods. Off J Eur Union 34:43 – 45

3. Summary and conclusion of the Joint FAO/WHO Expert Committee and Food Additives, 64th meeting, Rome, 8 -17 February 2005, JECFA/64/SC

4. European Commission (2009) Report on the third inter-laboratory comarison organised by rht community reference laboratory for polycyclic aromatic hydrocarbons, ISBN: 978ß92-79-11732-9, Joint Recearch Center

Acknowledgment

Thermo SCIENTIFIC